

MEMOIRS OF THE DEPARTMENT OF AGRICULTURE IN INDIA

ON THE INHERITANCE OF SOME CHARACTERS
IN WHEAT. I

BY

ALBERT HOWARD, M.A., A.R.C.S., F.L.S.

Imperial Economic Botanist

AND

GABRIELLE L. C. HOWARD, M.A.

Personal Assistant to the Imperial Economic Botanist



AGRICULTURAL RESEARCH INSTITUTE, PUSA

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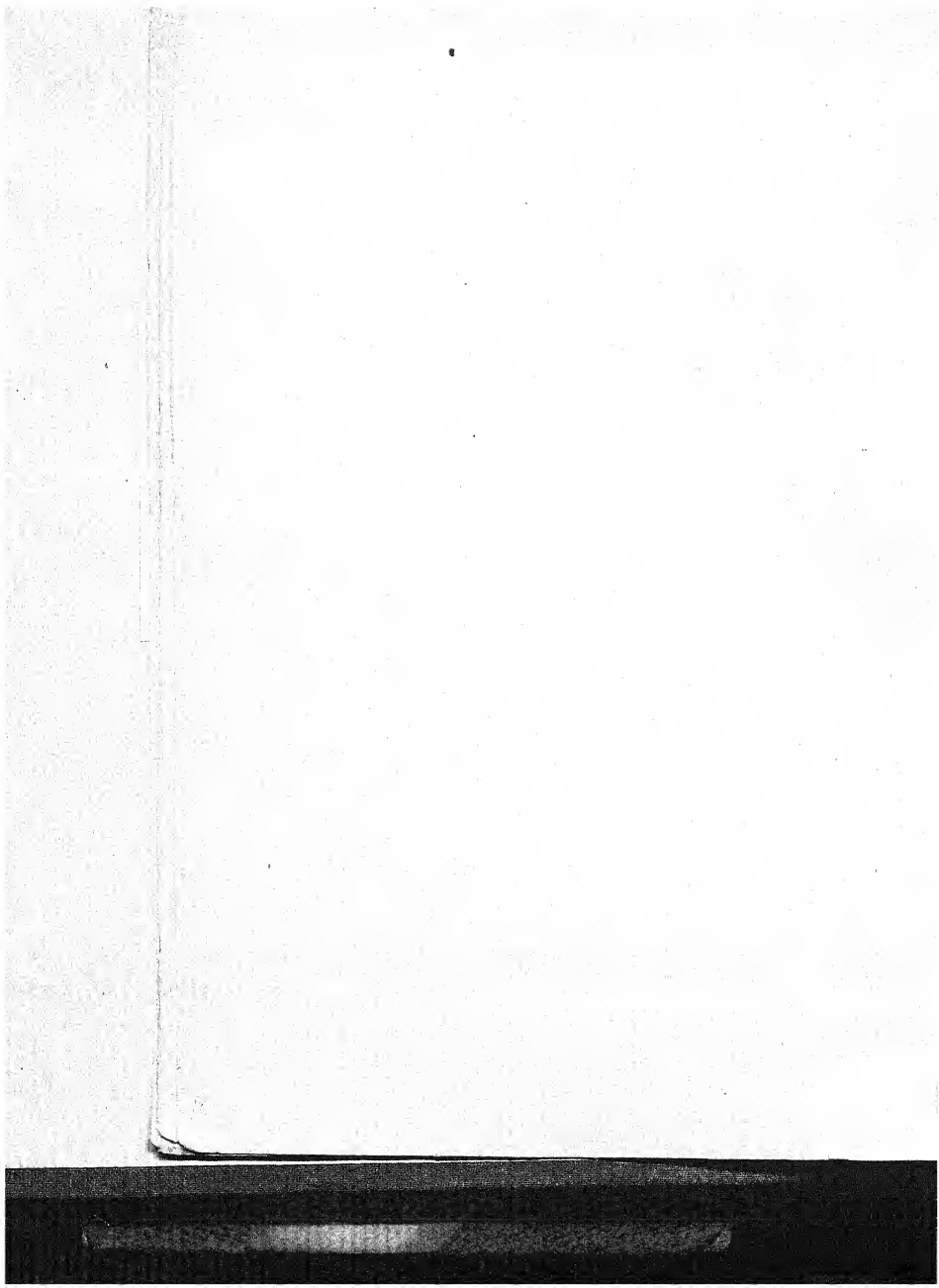
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I. INTRODUCTION.

THE investigations described in this paper form part of the work undertaken at Pusa during the years 1905 to 1912 in connection with the improvement of Indian wheat. The earlier practical results and those dealing with classification are to be found in former papers. The present account deals only with the information obtained on the laws governing the inheritance of the various characters in wheat and with the gametic factors which are involved in these characters.

The results relating to the red colour of the grain, in which the occurrence of several factors was demonstrated, were obtained some years ago and were about to be published when the admirable investigations of Nilsson-Ehle¹ at Svalöf were made public. This information was in consequence held over until the experiments dealing with bearding and felting could be included. The results obtained at Pusa both confirm and extend the work of Nilsson-Ehle

¹ Nilsson-Ehle, *Kreuzungsuntersuchungen an Hafer und Weizen*, Lund, 1909; *Kreuzungsuntersuchungen II*, Lund, 1911.

as to the complex nature of the characters in wheat and the existence of more than one factor in characters which to the eye appear simple.

Nilsson-Ehle, starting from the standpoint of Bateson's presence and absence hypothesis, has obtained very interesting results at Svalöf. He bases his conclusions on a study of wheat and oats, and the characters investigated in wheat were chaff colour, grain colour, ear form and resistance to yellow rust. His conclusions are summed up in the following paragraphs:—

1. Characters which to the eye appear simple, for example, the red colour of the grain or chaff, may be complex and may contain two or more factors. These factors may exist independently and each produces by itself a red colour and thus the red colour in chaff or grain, even when simple, is not always due to the same factor. When two red wheats are crossed, each containing several red factors, one of which is common to both, the result, in the second generation, is a series of red tones which, although distinct, defy accurate classification. Thus when the factors are numerous, and the differences between them are small, a continuous series of forms would result in the F_2 .

2. In the mode of inheritance there is no inherent difference between qualitative and quantitative characters. In such quantitative characters as resistance to rust or length of the internodes of the rachis the behaviour of the various generations from the cross indicate the existence of several factors. The difficulty in dealing with the inheritance of physiological characters such as rust resistance and with characters concerned with size is the want of an analyser, that is, a wheat in which the particular character is totally absent. In studying the inheritance of red colour factors, a white wheat acts as an analyser. It is, however, impossible to use a wheat without height or size in investigating characters dealing with the dimensions of organs. Similarly, there is no absolutely rust resistant wheat which can act as an analyser for the study of the factors involved in this character. Both qualitative and quantitative characters may be complex and may be made up of several factors which are inherited independently.

3. The influence of environment is of considerable importance in the study of inheritance when the factors differ from each other by small amounts. The number of homozygotic forms which are possible is expressed by the formula 2^n , where n is the number of factors. Thus, if there are four factors, 16 homozygotic forms are possible. It is obvious that when the total number of factors in any one character is large the types of wheat will differ from each other very slightly. Nevertheless these differences will be inherited. In studying this inheritance, however, environmental differences supervene, and these latter may be greater than the inherited differences. For example, in the study of inheritance of height in wheat the environmental differences may be greater than and so mask the inherited variations.

4. The existence of so many factors and the occurrence of natural crossing^{1 2 3} are sufficient to account for the complexity of botanical varieties in wheat and for the existence of a large number of types which breed true and which differ from each other very slightly. Even a small number of factors by recombination among themselves is sufficient to produce a large number of types. Many of the characters of wheat have already been shown to owe their existence to several factors. Further work will doubtless increase the number of these factors. (In the investigations described in the present paper relating to felted and smooth chaff and to bearded and beardless wheats it is shown that at least four and possibly more factors are involved in each of these characters.) The various combinations of these factors among themselves would give a relatively enormous number of different wheats.

5. The complexity of the characters in wheat renders it more than ever necessary that all studies in inheritance should be made with pure lines, that is, with cultures grown from a single parent plant. Such cultures, however, even if they breed true in all visible characters, may not necessarily be homozygotic, especially in physiological characters. In the present state of knowledge,

¹ Kiessling, *Fähling's land. Zeitung*, Bd. 57, 1908, s. 737.

² Nilsson-Ehle, *l.c.*, 1909, s. 16.

³ Howard and Howard, *Mem. Dept. Agr. in India* (Bot. series), Vol. III, No. 6, 1910.

however, pure lines are the best material available for the work.

Three series of crosses with Indian wheats are involved in the investigations described in this paper, and in all cases the work was started from pure lines. The first was made at Lyallpur in 1907 between various pure lines of Punjab wheats. The first and second generations of these crosses were grown at the Cawnpore Experiment Station¹ and the third generation at Pusa. In this series of crosses the F_1 plants of each cross were mixed, while the F_2 and F_3 plants were kept separate and sown and reaped plant by plant. The second series of crosses was made at Pusa with various pure lines obtained from local and other wheats which had been under observation for some years. In this series the F_1 plants were kept separate, so that the full history of each cross can be traced from each cross-fertilized seed. The third series of crosses was made at Cambridge in the summer of 1910, and the F_1 generation was grown partly at Pusa and partly at Cawnpore in the wheat season of 1910-11, while the second generation was grown at Pusa in 1911-12. The principal object of this latter series was to cross European and American rust resistant wheats with various Indian lines of high grain quality with the object of securing hybrids of use in India, characterised by higher rust resistance and stronger straw than those at present grown. American Club, which was used by Biffen at Cambridge as a parent resistant to yellow rust, when grown at Pusa proved to be very rust resistant under Indian conditions, although it was a late form and did not complete its growth period till well into the hot weather when forms like Einkorn are always attacked by black rust (*P. graminis*) sooner or later. Its late period of maturity prevented any crossing with Indian wheats at Pusa, so it was decided to attempt the work at Cambridge where the Indian parents were sown for us by Professor Biffen as spring wheats in 1910. The Indian wheats developed very feebly under English conditions, but sufficient pollen was

¹ For facilities at the Cawnpore Experiment Station we take this opportunity of acknowledging our indebtedness to Mr. H. Martin Leake, Economic Botanist to the United Provinces.

obtained for crossing on to various English and American parents, such as American Club and Red Fife. We take this opportunity of expressing our thanks to Professor Biffen for facilities given for the hybridization work at Cambridge and for the sowing and care of the Indian cultures in 1910.

In the conduct of hybridization work on wheat under sub-tropical conditions several difficulties have to be overcome which are not experienced in Europe and America. At Pusa, wheats have to be grown on the moisture stored up in the subsoil during the previous monsoon and the rainfall after sowing time is insignificant and may be nothing at all. All the generations have to be raised as a dry crop, and the sowing must be carried out without loss of moisture. Irrigation tends to prolong the growth period of plants sown singly and to bring on rust. Any lengthening of the growth period of wheat is a great disadvantage in India as the plants are bound to be dried up by the hot winds before ripening can take place. The great difficulty is to grow the single seeds under conditions comparable with those obtaining in a field crop. For this it is necessary that the seed should be sown at the proper depth and the soil consolidated afterwards. Dibbling in the seed or sowing in furrows made by hand ploughs is not satisfactory as the plants do not root properly and do not stand up during the windy weather previous to harvest. Such plants also seem to develop a shallow root-system and require a considerable amount of irrigation water to maintain them. The best method so far found is to sow the seeds singly in furrows made by the plough, to cover them by hand and to consolidate the soil by a roller. This results in well rooted plants, but the disadvantage of too much space for each plant and consequently the prolongation of the growth period into the hot weather, with the practical certainty of black rust and premature ripening, still remains. Until the various hybrids forms are fixed and sufficient seed has been obtained for a plot sown in the ordinary manner, it is impossible to say with certainty how they will behave under field conditions. Consequently hybridization work in India involves a very considerable amount of work in the testing of the new forms in plots.

Another disadvantage in India concerns the examination and storage of the harvest. The wheat season is followed shortly by the monsoon when the damp hot air speedily spoils the wheat and brings on the conditions under which its complete destruction by weevils and moths is only a matter of a very few days. It is not possible therefore to work on the material of the previous harvest after June 1st, so that it frequently happens that the material of one harvest cannot be worked through before sowing time comes round. The crop therefore may have to be stored in safely over at least two monsoons. This is done by putting away the bundles of ears of the single plants, when thoroughly dried, in air-tight iron cylinders where they can be kept at least three years without damage or loss of germinating power.

India, however, possesses one great advantage in this work, namely, cheap labour. The cost is only about ten per cent. of that of Europe, so that it is a simple matter to conduct the work on a large scale and to sow and reap at least 1,000 plants of each cross in the second generation. It will be seen that in crosses in which one parent contains a three factor character, this number is by no means excessive if the inheritance is to be worked out in anything approaching thoroughness.

II. FELTED AND SMOOTH CHAFF.

THE inheritance of the felting of chaff in wheat has not previously been worked out in any great detail. Biffen¹ states in regard to this character "The felted or velvety character is dominant, the glabrous recessive." In many cases the 3 : 1 ratio was obtained in the F_2 , but in the case of Rivet crosses a series was met with in the F_1 and a ratio of 223 : 116 in the F_2 . In the F_3 , however, the splitting was simple. Tschermak², in a more recent account, states that felting of the glumes prevails over smoothness, but that in many crosses the F_1 is plainly intermediate and the splitting in the second generation is an impure one in which the various stages of felting stand to smooth in the ratio 3 : 1. In one case, where Rivet bearded was crossed with Saxon red, Rimpau mentions the total disappearance of felting in the hybrid. Pitsch⁴ found in Felted Essex \times felted *Rouge inversable* some smooth progeny in the F_2 . The Svalöf results agree with the Mendelian scheme and ratios of 2.7 to 1, 3.7 to 1 and 2.8 to 1 were obtained. In all cases the first generation was felted and in one case in the second generation of smooth. Svalöf Extra Square head crossed with a felted wheat one intermediate plant was found.

COMPLEX FELTING.

In this section cases are dealt with in which the felting is due to more than one factor. The first four crosses with a felted wheat were made at Lyallpur in 1907 and in all cases a pure line selected from Punjab Type 9, a red wheat with densely felted chaff, was employed as one of the parents. This was chosen on account of its high yielding power and strength of straw while the other parents

¹ Biffen, *Jour. of Agr. Science*, 1, 1905, p. 24.

² Tschermak, *Die Züchtung der land. Kulturpflanzen*, Bd. IV, 1910; *Zeit. f. d. land. Versuchswesen in Oesterreich*, 1901, s. 1029.

³ Rimpau, *Land. Jahrbücher*, 1891.

⁴ Pitsch, *Deutsche Land. Presse*, 1899.

were white wheats with relatively poor straw. Later, a fifth cross, with the same felted parent, was made at Pusa and the second generation was raised in 1911-12. The five crosses, in each of which the reciprocal was made, were as follows:—

1. Punjab Type 9 (β) \times Punjab Type 16.
2. Punjab Type 9 (β) \times Mozaffarnagar white.
3. Punjab Type 9 (β) \times Punjab Type 25.
4. Punjab Type 9 (γ) \times Punjab Type 13.
5. Punjab Type 9 (α) \times Line H II 4.

α , β , γ , denote similar pure lines from Punjab Type 9.

In all these cases the F_1 was intermediate and in the second generation the ratio of felted to smooth was 15 : 1. The felted plants formed a series and there appeared to be every possible gradation between plants like the felted and smooth parents. All that could be done was to group the plants round arbitrary standards, but many cases arose where a plant could, with equal justice, be placed in either of two groups. The ratios, felted to smooth, obtained in the second generation of these crosses are given in the following table:—

TABLE I.

The F_2 generation of crosses between complex felted and smooth parents.

Cross.	No. of plants.	Felted.	Smooth.	Ratio felted to smooth.
Punjab Type 9 (α) $\varnothing \times$ H II 4 σ	(1) 187	127	10	12.7 : 1
" " "	(2) 190	176	14	12.6 : 1
" " "	(3) 185	171	14	12.2 : 1
H II 4 $\varnothing \times$ Punjab Type 9 (α) σ	(4) 117	108	9	12.0 : 1
" " "	(5) 175	166	9	18.4 : 1
Total	804	748	56	13.4 : 1
Punjab Type 9 (β) $\varnothing \times$ Punjab Type 16 σ (a)	601	562	39	14.4 : 1
" " "	(b) 587	550	37	14.8 : 1
Punjab Type 9 (β) $\varnothing \times$ Moz. White σ (a)	708	658	50	13.2 : 1
" " "	(b) 442	411	31	13.2 : 1
Punjab Type 9 (β) $\varnothing \times$ Punjab Type 25 σ (a)	1,017	961	56	17.1 : 1
" " "	(b) 603	567	36	15.7 : 1
Punjab Type 9 (γ) $\varnothing \times$ Punjab Type 13 σ	965	917	48	19.1 : 1
Total of all crosses with Type 9	5,727	5,374	353	15.2 : 1
Expectation		5,390	358	

a and b denote the numbers obtained by different observers

These numbers suggested the existence of two factors in the felted parent and on examination under the binocular dissecting microscope it was observed that the hairs on the chaff of the felted parent were of two kinds—long silky and short hairs. The intermediate first generation showed on examination under the microscope that both kinds of hairs were present, but both were diminished in length and in density. From the fact that the ratio 15:1 was obtained in the F_2 in all the crosses with smooth chaffed wheats and from the appearance of the F_1 generation it appeared probable that these two sorts of hairs were each due to a single factor and were inherited independently. In this case, on Bateson's presence and absence hypothesis, denoting the long factor by LL and the short factor by SS the scheme up to the F_2 generation would be represented by the following:—

$$\begin{array}{rcccl}
 \text{SS LL} \times \text{ss ll} & \dots & \dots & \dots & \text{Parents.} \\
 | & & & & \\
 \text{Ss Ll} & \dots & \dots & \dots & \text{First generation.} \\
 | & & & & \\
 \text{SS LL} + 4 \text{Ss Ll} + 2 \text{Ss lL} + 2 \text{SS Ll} & & & & \\
 + \text{sS LL} + 2 \text{sS Ll} + \text{SS ll} + 2 \text{Ss ll} + \text{ss ll} & \} & \text{Second generation.}
 \end{array}$$

On this assumption the appearance of these various classes in the F_2 generation and the nature of the progeny in the F_3 would be those shown in the following table:—

F_2	F_3
(1). SS LL both kinds of hairs complete.	Constant like the F_2 .
(2). Ss Ll both sets partly developed as in F_1 .	Series like the F_2 with felted to smooth in the ratio 15:1.
(3). Ss LL long factor complete, short factor partial.	All variously felted (SS LL + 2 Ss LL + ss LL).
(4). SS Ll short factor complete, long partial.	All variously felted (SS LL + 2 SS Ll + SS ll).
(5). ss LL long factor only complete, short absent.	Constant like the F_2 .
(6). ss Ll long factor partial, short absent.	Felted: smooth :: 3:1 (ss LL + 2 ss Ll + ss ll).
(7). SS ll short factor only complete, long absent.	Constant like the F_2 .
(8). Ss ll short factor only partial, long absent.	Felted: smooth :: 3:1 (SS ll + 2 Ss ll + ss ll).
(9). ss ll smooth.	Constant like F_2 .

Although the existence of the various groups in the F_2 could be demonstrated by means of the binocular microscope it proved impossible to analyse this generation completely without an undoubted

representative of each class as a standard. This portion of the work was therefore deferred and the F_2 was first studied as thoroughly as possible in two of the four crosses—Nos. 2 and 3 in the list on page 8. A very large number of plants in the F_2 were selected by eye as representative of the whole series and in addition all the smooth specimens were grown. All the plants were sown seed by seed and the resulting F_2 cultures were reaped plant by plant. The individuals of each of these F_2 cultures were then examined separately under the microscope. The results obtained in this examination in cross No. 2 are given in detail in tables II to V:—

TABLE II.

The F_2 generation of a cross between a complex felted and a smooth plant.

FELTING IN THE F_2 GENERATION.												
Plant No.	Felting in F_2 parent.	No. of plants in P_2 .	Long and short hairs complete, SS LL.	Long and short hairs partial, Ss LL.	Long hairs complete, short partial, Ss LL.	Long hairs partial, short complete, SS LL.	Long hairs complete, ss LL.	Long hairs partial, ss LL.	Short hairs complete, SS ll.	Short hairs partial, Ss ll.	Smooth, ss ll.	Ratio.
47	Long hairs complete, short hairs partial (Ss LL).	66	14	...	39	...	13	
51	do.	57	14	...	38	...	15	
128	do.	73	17	...	35	...	21	
130	do.	72	16	...	41	...	15	
150	do.	68	16	...	39	...	13	
191	do.	79	20	...	39	...	20	
258	do.	72	22	...	32	...	18	
368	do.	70	22	...	34	...	14	
392	do.	58	17	...	28	...	13	
507	do.	69	21	...	38	...	10	
509	do.	100	33	...	53	...	14	
586	do.	78	23	...	45	...	10	
597	do.	86	22	...	44	...	20	
713	do.	48	12	...	27	...	9	
700*	do.	62	18	...	31	...	13	
789	do.	83	21	...	39	...	23	
818	do.	80	18	...	45	...	17	
861	do.	70	20	...	34	...	16	
863	do.	55	16	...	28	...	11	
894	do.	72	22	...	33	...	17	
889	do.	77	21	...	44	...	12	
911	do.	88	23	...	43	...	22	
995	do.	53	13	...	27	...	13	
Total	...	1,686	441	...	846	...	349	
Expectation	...		408	...	818	...	408	
152	Long hairs partial, short complete (SS LL).	98	27	46	25	
272	do.	74	22	39	13	
338	do.	73	15	39	19	
412	do.	74	23	40	11	
579	do.	143	46	81	16	
940	do.	87	29	40	18	
975	do.	95	11	53	31	
Total	...	614	173	338	138	
Expectation	...		161	322	161	
9	Long hairs complete (ss LL).	67	67	Uniformly felted.
39	do.	41	41	"
65	do.	75	75	"
185	do.	65	65	"
681	do.	79	79	"
724	do.	76	76	"
771	do.	105	105	"
Total	...	467	467	"
Expectation	467	"

TABLE III.

The F_3 generation of a cross between a complex felted and a smooth plant.—(Continued.)FELTING IN THE F_3 GENERATION.

Plant No.	Felting in F_3 generation.	No. of plants F_3 .	Long and short hairs complete, SS Ll.	Long and short hairs partial, Ss Ll.	Long hairs complete, short partial, Ss Ll.	Long hairs partial, short complete, SS Ll.	Long hairs complete, ss Ll.	Long hairs partial, ss Ll.	Short hairs complete, SS ll.	Short hairs partial, Ss ll.	Smooth, ss ll.	Ratio felted to smooth.
10	Long hairs partial (ss Ll).	77	21	37	19	3:1:1
16	do.	88	19	42	27	2:3:1
44	do.	77	20	43	14	4:5:1
45	do.	81	18	41	22	2:7:1
53	do.	69	28	32	9	6:7:1
150	do.	73	18	40	15	3:9:1
193	do.	69	20	31	18	2:8:1
211	do.	88	23	39	26	2:4:1
218	do.	77	19	34	24	2:2:1
256	do.	97	24	49	24	3:0:1
285	do.	73	19	39	15	3:9:1
426	do.	89	22	41	26	2:4:1
435	do.	133	41	53	39	2:4:1
488	do.	110	25	56	29	2:8:1
510	do.	114	32	51	31	2:7:1
523	do.	78	22	37	19	3:1:1
589	do.	108	17	60	31	2:5:1
590	do.	81	22	38	21	2:9:1
592	do.	92	34	38	20	3:6:1
599	do.	69	12	34	23	2:4:1
605	do.	94	22	44	28	2:4:1
632	do.	95	26	42	27	2:5:1
636	do.	82	17	37	28	1:9:1
696	do.	128	30	68	30	3:3:1
698	do.	86	22	44	20	3:3:1
707	do.	70	19	36	15	3:7:1
726	do.	115	25	54	36	2:2:1
733	do.	69	22	36	11	5:3:1
739	do.	124	36	59	29	3:3:1
791	do.	71	15	41	15	3:7:1
831	do.	88	23	45	20	3:4:1
833	do.	118	22	61	35	2:4:1
854	do.	102	31	49	22	3:6:1
873	do.	90	27	49	17	4:3:1
892	do.	140	39	75	26	4:4:1
948	do.	117	24	62	31	2:5:1
965	do.	108	25	54	23	2:7:1
969	do.	68	13	33	29	3:1:1
983	do.	97	22	37	28	2:5:1
985	do.	63	20	33	10	5:3:1
1004	do.	113	30	61	22	4:1:1
Total	...	3,781	966	1,862	953	2:97:1
Expectation							945-25	1890-5			945-25	

The F₃ generation of a cross between a complex felted and a smooth plant.—(Continued.)

Expectation

The F₂ generation of a cross between a complex felted and a smooth plant.—(Concluded.)

FELTING IN THE F ₂ GENERATION.												
Piant No.	Felting in F ₂ parent.	No. of plants in F ₂ .	Long and short hairs complete. SS LL.	Long and short hairs partial. Ss Ll.	Long hairs complete, short partial. Ss LL.	Long hairs partial, short complete. SS Ll.	Long hairs complete. ss LL.	Long hairs partial. ss Ll.	Short hairs complete. SS ll.	Short hairs partial. Ss ll.	Smooth. ss ll.	Ratio felted to smooth.
58	Short hairs partial (Ss ll).	82	19	43	20	3:1:1
77	do.	128	27	77	24	4:3:1
114	do.	79	14	49	16	3:4:1
473	do.	105	29	23	3:4:1	
560	do.	54	12	29	13	3:1:1
651	do.	90	18	39	33	1:7:1
702	do.	72	25	33	14	4:1:1
711	do.	81	20	37	24	4:1:1
728	do.	86	19	41	26	2:3:1
744	do.	55	15	27	13	3:2:1
770	do.	123	30	65	33	2:9:1
796	do.	97	26	43	28	2:5:1
853	do.	65	13	33	17	2:3:1
875	do.	68	15	32	21	2:2:1
922	do.	93	6	12	5	3:6:1
984	do.	121	31	64	26	3:6:1
1,026	do.	94	25	48	21	3:5:1
Total		1,428	344	727	357	3:0:1
Expectation									357	714	357	
15	Short hairs complete (SS ll).	95	95	Uniformly felted.
223	do.	71	71
243	do.	81	81
260	do.	81	81
373	do.	102	102
665	do.	53	53
737	do.	96	96
993	do.	74	74
Total		658	658
Expectation									658			
67	Short and long hairs complete (SS LL).	99	99	Uniformly felted.
79	do.	112	112
106	do.	104	104
119	do.	103	103
138	do.	99	99
153	do.	?	all
184	do.	63	63
463	do.	77	77
513	do.	?	all
517	do.	?	all
554	do.	66	66
582	do.	91	91
763	do.	103	103
820	do.	81	81
844	do.	81	81
859	do.	80	80
973	do.	67	67
990	do.	67	67
Total		1,293	1,293
Expectation			1,293									

It will be seen that the F_3 cultures could be divided into the following classes :—

1. Cultures uniformly and densely felted derived from $SSLL$.
2. Cultures uniformly and lightly felted derived from $ssLL$ and $SSll$.
3. Cultures with all plants felted, but not uniformly, derived from $SSLl$ and $SsLL$.
4. Cultures with felted and smooth plants in the ratio 15 : 1 derived from $SsLL$.
5. Cultures with felted and smooth plants in the ratio 3 : 1 derived from $Ssll$ and $s s L l$.
6. Cultures all smooth.

In dealing with the offspring obtained in the F_3 generation from the various F_2 plants, it was an easy matter to separate classes 4, 5 and 6 from the others and from each other. It was also comparatively simple to separate classes 1, 2 and 3 from each other by the aid of the microscope. By examining a large number of plants in each culture it was not difficult to see whether the felting was uniform or not, and whether one or both kinds of hairs were present. The distinction between the two constituents of class 3, namely, the cultures derived from plants of the gametic constitution $SsLL$ and $SSLl$ was not quite so simple. As was shown on page 9 plants of the constitution $SsLL$ give in the F_3 $SSLL + 2SsLL + ssLL$ (long hairs completely developed), while $SSLl$ gives $SSLL + 2SSLl + Ssll$ (short hairs completely developed). The best means of distinguishing these lies in the well marked difference between plants represented by $s s L L$ and $S S l l$. Glumes with the long hairs only and complete are easily distinguished under the microscope from those possessing the short hairs only, both by the length and also by the density of the hairs (the long hairs are more sparsely distributed than the short). On this rests the separation of the constituents not only in class 3 but also in class 5. Had the two kinds of hairs been more alike, these classes could not have been subdivided.

It will be seen on examining the tables of the F_3 generation of this cross that the determination of the various classes of felting

involved the microscopic examination of nearly 15,000 plants and that considering the difficulty of the work the numbers obtained correspond very fairly well with expectation. A considerable number of cultures were examined in the class (S s L l), which gave the 15 : 1 ratio, but time did not permit of the detailed examination of more than 831 plants belonging to 10 cultures. The ratio felted to smooth, however, was worked out for 3,912 plants belonging to 35 more cultures. There can be little doubt that had time permitted for the examination of these 3,912 plants they would have confirmed the ten cultures of this class which were worked out in detail.

The complete study of the F_3 generation enabled us to examine the 1,017 plants of the F_2 . After the examination of the F_3 it was easy to obtain a representative plant of each of the eight classes of differently felted plants which occurred in the F_2 . By mounting a glume of each of these representatives on a slide a set of standards was obtained by the aid of which it was possible to divide the F_2 material into its constituents. A glume of each of the 1,017 F_2 plants was compared with the standards and the class was determined by inspection. The results are given in table VI and it will be seen that the figures obtained compare fairly well with expectation. Even with these standards it was sometimes difficult to determine the proper class to which an individual belonged. For example, in distinguishing plants of the gametic constitution S S l l and s s L l the difference is mainly one of density of the felting, the partially developed long hairs being of almost the same length as the completely developed short ones. A similar difficulty arose with plants represented by S S L L and S s L L.

TABLE VII.

The F_3 generation of another cross between complex felted and smooth parents.

FELTING IN THE F_3 GENERATION.												
Plant No.	Felting in F_2 parent.	No. of plants in F_3 .	Long and short hairs complete, SS LL.	Long and short hairs partial, Ss LL.	Long hairs complete, short partial, Ss LL.	Long hairs partial, short complete, SS LL.	Long hairs complete, ss LL.	Long hairs partial, ss LL.	Short hairs complete, SS ll.	Short hairs partial, Ss ll.	Smooth, ss ll.	Ratio.
10	Long and short hairs complete (SS LL).	95	95	Uniformly felted, do.
15	do. ...	95	95	do.
	Total ...	190	190	
30	Long and short hairs partial (Ss Ll).	95	4	22.7 : 1
72	do. ...	59	1	58.0 : 1
86	do. ...	183	27	5.8 : 1
101	do. ...	61	6	9.1 : 1
147	do. ...	91	8	10.7 : 1
166	do. ...	93	6	14.5 : 1
199	do. ...	19	1	18.0 : 1
285	do. ...	98	6	15.2 : 1
315	do. ...	89	3	28.7 : 1
322	do. ...	30	6	13.0 : 1
402	do. ...	85	8	9.6 : 1
470	do. ...	28	4	8.5 : 1
689	do. ...	78	9	12.0 : 1
	Total ...	1,022	86	10.9 : 1
	Expectation										63.9	
51	Long hairs complete, short partial (Ss LL).	100	gave felted progeny only (SS LL, Ss LL and ss LL)							All felted but not uniform.
21	Long hairs partial, short complete (SS Ll).	44	gave felted progeny only (SS LL, Ss LL and SS ll)							do.
202	do. ...	37								do.
358	do. ...	53								do.
238	Long hairs partial (ss Ll).	80	62	18	3.4 : 1
692	do. ...	83	65	18	3.6 : 1
	Total ...	163	127	36	3.5 : 1
	Expectation						122.5				39.75	
244	Short hairs complete (SS ll).	37	37	All uniformly felted, do.
264	do. ...	78	78	do.
278	do. ...	(7)	all	do.
281	do. ...	118	all	do.
367	do. ...	(2)	all	do.
466	do. ...	78	78	do.
	Total ...	314 + 2	all	do.
52	Short hairs partial (Ss ll).	93	74	19	2.3 : 1
251	do. ...	99	69	30	2.3 : 1
323	do. ...	85	66	19	3.5 : 1
372	do. ...	93	68	25	2.7 : 1
451	do. ...	88	66	22	3.0 : 1
	Total ...	458	343	115	3.0 : 1
	Expectation									243.5	114.5	

TABLE VI.

The analysis of the F_2 generation of crosses between complex felted and smooth parents.

Cross.		No. of plants,	Long and short hairs complete, SS LL,	Long and short hairs partial, Ss Ll,	Long hairs complete, short partial, Ss LL,	Long hairs partial, short complete, SS Ll,	Long hairs complete, ss LL,	Long hairs partial, ss Ll,	Short hairs complete, SS ll,	Short hairs partial, Ss ll,	Smooth, ss ll,
Punjab Type 9 ♀ ×	H II ₄ ♂	190	13	50	19	19	10	23	14	28	14
Expectation.			11.9	47.6	23.7	23.7	11.9	23.7	11.9	23.7	11.9
Punjab Type 9 ♀ ×	Punjab Type 25 ♂	1,017	70	261	115	140	79	107	72	117	56
Expectation.			63.5	254	127	127	63.5	127	63.5	127	63.5
TOTAL.		1,207	83	311	134	159	89	130	86	145	70
Expectation.			75.4	301.7	150.8	150.8	75.4	150.8	75.4	150.8	75.4

The cultures of the other cross on Punjab Type 9 which had been continued to the F_3 generation, namely, that on Mozaffarnagar white, were not completely examined owing to want of time. This cross was not dealt with in such detail as in the previous case, but sufficient cultures were examined to show that in this case also the inheritance was the same as in the former. The results are given in table VII:—

The unravelling of the F_2 in the first cross now enabled us to deal with another cross (No. 5 on page 8) in which a pure line from Punjab Type 9 was used as the felted parent. In this case the F_2 material was dealt with direct as the F_2 generation of this cross has not yet been grown. One hundred and ninety F_2 plants were examined under the microscope and the glumes were compared with the set of standards. The numbers obtained are given in the first portion of table VI above, and they are very close to those expected. In this cross the smooth parent was a pure line known as H II₂.

Thus it has been demonstrated that in the case of Punjab Type 9 the felting is composed of two kinds of hairs, each of which can exist separately and is inherited independently of the other.

Another case of complex felting, in which two kinds of hairs are concerned, was met with in the second series of crosses made at Pusa. In this case the felted parent was a pure line from the durum wheat known as Pusa 20, the smooth parent being the line of common wheat known as H III₂. In the F_1 the felting was intermediate, both factors being present, but both reduced in length and density. In the F_2 the figures obtained were 481 felted to 36 smooth pointing to the 15 : 1 ratio. Only 517 plants were grown in the F_2 and the felted individuals formed a series as before. The details so far obtained with this cross are given in the following table :—

TABLE VIII.

The F_2 generation of a cross between a complex felted durum and a smooth common wheat.

Cross.	No. of plants.	Felted.	Smooth.	Ratio felted to smooth.
Pusa 20 ♀ × H III ₂ ♂ (1) ...	61	58	3	19.3 : 1
" " (3) ..	119	112	7	16.0 : 1
" " (4) ...	73	66	7	9.4 : 1
" " (5) ...	98	90	8	11.3 : 1
H III ₂ ♀ × Pusa 20 ♂ (1) ...	54	50	4	12.5 : 1
" " (2) ...	59	55	4	13.8 : 1
" " (4) ...	53	50	3	16.7 : 1
TOTAL ...	517	481	36	13.4 : 1
Expectation		484	32.3	

A marked difference between the short hairs in Pusa 20 and those in Punjab Type 9 was noticed. In this cross in the wheats of the gametic constitution $S s 1 1$ the short hairs almost disappear and it is only with the greatest difficulty that these forms can be distinguished from those with smooth glumes. This extreme reduction of short hairs in the F_1 was met with again in one of the cases of simple felting referred to below. It would therefore appear probable that the short hairs in Pusa 20 are not due to the same factor as those in Punjab Type 9. It is also improbable, judging by appearance, that the long hairs in both these wheats are identical, the long hairs in Punjab Type 9 being much denser and longer than those in Pusa 20. It is therefore probable that we have four different kinds of hairs, each due to an independent factor and inherited separately, but this can only be proved by further experiments. In Pusa 20 both the kinds of hairs do not differ very widely and it will be much more difficult to analyse the F_2 generation than in the crosses with Punjab Type 9.

SIMPLE FELTING.

In two of the parents with felted chaff only one class of hairs was distinguished under the microscope, and in both cases the 3 : 1 ratio was obtained in the F_2 . The felted parents in these crosses were lines selected from common wheats and are described in the records as B XI 77 and Pusa 4. The hairs on these wheats, when directly examined, appeared to be different, and this was confirmed by their behaviour in the F_1 . In the F_1 from Pusa 4 the same phenomenon as in the crosses on Pusa 20 was observed, namely, the almost total disappearance of the hairs, while in the F_1 from B XI 77 the felting could be seen by the naked eye. If the felting factors in these two wheats are different, a cross between them would give smooth progeny in the F_2 . The actual experiment, however, has not yet been carried out. The details of the second generation of the above crosses are given in the following table :—

TABLE IX.

The F₂ generation of crosses between simple felted and smooth parents.

CROSS.		No. of Plants.	FELTED.		Smooth.	Ratio.
			Fully.	Lightly.		
BXI 77 ♀ × HI ₂ ♂	(1)	194	48	100	46	1:2.2:1
" "	(2)	227	56	117	54	1:2.2:1
" "	(3)	233	59	95	79	7:1.2:1
HI ₂ ♀ × BXI 77 ♂	(5)	253	59	124	70	8:1.8:1
TOTAL		907	222	436	249	9:1.8:1
			658		249	2.64:1
<i>Expectation</i>	...		226.76	453.6	226.76	
			680		227	
American Club ♀ × Pusa 4 ♂	(1)	156	32	77	47	7:1.6:1
" "	(2)	177	34	95	48	7:2.0:1
" "	(3)	195	43	105	47	9:2.2:1
" "	(4)	124	27	64	33	8:1.9:1
" "	(5)	193	47	98	48	1:2.0:1
" "	(6)	216	51	109	56	9:1.9:1
TOTAL		1,061	234	548	279	8:2.0:1
			782		279	2.8:1
Biffen's hybrid ♀ × Pusa 4 ♂	(1)	158	37	79	42	9:1.9:1
" "	(2)	178	48	88	42	1.1:2.1:1
" "	(3)	110	26	56	28	9:2.0:1
" "	(4)	115	36	47	32	1.1:1.5:1
" "	(5)	221	55	111	55	1:2.0:1
" "	(6)	197	49	103	45	1.1:2.3:1
TOTAL		979	251	484	244	1:2.0:1
			735		244	3:1
Total of both crosses with Pusa 4		2,040	485	1,032	523	
			1,51		523	2.9:1
<i>Expectation</i>	...		1,530		510	

Thus it has been shown that the felting on the chaff of wheat is due to the presence of different kinds of hairs, one or more of which may be present in the same wheat. In all our investigations up to the present each kind of hair has been shown to be due to a single factor and to be inherited independently of any other kind of hair present. The microscopic comparison of the wheats Pusa 20 and Punjab Type 9, and also the breeding experiments make it probable that though each wheat contains two kinds of hairs differing in length, none of the hairs are alike. This gives at least four kinds of hairs and four different factors in two wheats only.

These results led to the examination under the microscope of a large number of other felted wheats, when some interesting observations were made. It was found that the differences in the length and density of the hairs composing the felting are more numerous than would be supposed, and it was found without exception that all densely felted wheats contained two different kinds of hairs, one of which was long and to which was due the densely felted appearance. A large number of macaroni wheats from all over India were examined and in all cases two kinds of hairs were present. With the exception of Pusa 20 and Sindhi of Partabgarh, all the Indian durums examined possess a peculiar long silky tomentose type of hair as well as short hairs. These long hairs are absolutely distinct from the long hairs of Punjab Type 9. As no breeding experiments have been carried out with these wheats, it is impossible to say whether these hairs are intensified forms of other long hairs or whether, as is more probable, they are due to an entirely new factor. In the common wheats examined the felting was found to be simple, but in these cases the different wheats were found to possess hairs of different length. It is probable, in the light of the experience already obtained, that these will be found to be different and to be due to several factors.

As an example of the diversity of the nature of the felted chaff in wheat, the results of an examination of the wheats of the Punjab may be given. In 1908, the wheats of this Province were shown to belong to 25 distinct agricultural types among which there are 8 felted kinds.

Punjab Type 1. ¹	<i>T. durum</i>	Two kinds of hairs one short, one very long and silky.
Punjab Type 2.	<i>T. durum</i>	Same as Type 1.
Punjab Type 5.	<i>T. compactum</i>	Short hairs only.
Punjab Type 8.	<i>T. vulgare</i>	Long hairs only, but these are not nearly as long or as silky as those on the macaroni wheats.
Punjab Type 9.	"	Two kinds of hairs, long and short; the long hairs quite different (shorter and less silky) than those on Types 1 & 2. The long hairs on this type may be the same as those in type 8.
Punjab Type 17.	"	Long hairs only as in Type 8.
Punjab Type 18.	"	Short hairs only present, but these are different in appearance to the short hairs present in Type 9.
Punjab Type 19.	"	Short hairs only present. From their appearance these may be the same as those in Type 9.

The felting of the glumes of wheat is therefore a much more complex character than has hitherto been considered, and the total number of factors involved in this character is considerable and much larger than would be supposed. Considering that a microscopic examination of this character is sufficient to disclose several sorts of hairs, it is surprising the complex nature of felting has not been observed before. Possibly the explanation of this is to be found in the fact that double felting seems to be commoner in macaroni or wheats derived from these than in wheats of the Northern temperate regions where much of the wheat breeding work has been done.

On looking through our collection of European felted wheats two sets of hairs were distinguished in the following Rivet wheats:—

Blé Nonette de Lausanne.

Poulard d' Australie.

Pétanielle noire de Nice.

Blé de miracle.

In all the European common wheats with velvet chaff which we have examined only one set of hairs was observed in any wheat, but the kind of hairs in these wheats was not always the same.

¹ Howard & Howard, *Memoirs of the Dept. of Agr. in India* (Bot. Series), Vol. II, No. 7, 1908.

The only felted common wheat, in which we have so far found two sets of hairs, is Punjab Type 9.

The demonstration of the complex nature of felting is of importance from the systematic point of view. Wheats with different sorts of felting can no longer be placed in the same botanical variety and the present accepted classificatory schemes will need some enlargement. The final unravelling of this character, however, can only be done by breeding experiments a work of some magnitude.

III. GRAIN COLOUR AND CHAFF COLOUR.

GRAIN COLOUR.

THE inheritance of the red colour in wheat has been investigated in great detail by previous observers. Biffen¹ found that red was dominant over white and obtained the 3 : 1 ratio in the F₂. Tschermak² states that red, brown and violet grain colour prevail over white and that after an intermediate F₁ pure and impure splitting in the F₂ takes place in the ratio 3 : 1. Both Tschermak and Nilsson-Ehle found that in certain crosses between red parents white grained individuals were produced in the F₂ thereby showing that the factors in each red parent were not the same. The most detailed investigations on this character are those of Nilsson-Ehle³ at Svalöf, who in addition to the simple splitting observed by Biffen and others, found ratios of 15 : 1 and 63 : 1 in the F₂, which were proved to be due to the presence of two and three factors respectively in the red parent. Each of these factors was found to be capable by itself of producing the red colour. These red parents with multiple grain colour factors were selected from old country varieties and were found to be exceedingly hardy and to be less liable to sprouting in the ear than white forms.

In our experiments on the inheritance of the red colour of the grain we have found that the F₁ is always red, but the tone of colour is intermediate and lighter than that of the red parent. In the F₂ the following ratios of red to white were obtained.

¹ Biffen, *l.c.*

² Tschermak, *l.c.*

³ Nilsson-Ehle, *l.c.*

TABLE X.

The F₂ generation of crosses with single and double red factors in the red parent.

CROSS.	No. of Plants.	GRAIN COLOUR.		RATIO.
		Red.	White.	
Biffen's hybrid ♀ × Pusa 4 ♂ (1)	153	110	43	2.6:1
" " (2)	176	140	36	3.9:1
" " (3)	109	87	22	3.9:1
" " (4)	112	80	23	3.8:1
" " (5)	221	179	42	4.2:1
" " (6)	193	141	54	2.6:1
TOTAL	966	746	220	3.4:1
Expectation		724.5	241.5	
B XI 77 ♀ × H 12 ♂ (1)	194	139	55	2.5:1
" " (2)	227	177	50	3.5:1
" " (3)	233	183	50	3.6:1
H 12 ♀ × B XI 77 ♂ (5)	233	190	63	3.0:1
TOTAL	907	689	218	3.2:1
Expectation		680.25	226.75	
Punjab Type 9 ♀ (α) H II ₄ ♂ (1)	137	119	18	6.6:1
" " (2)	189	183	6	30.5:1
" " (3)	185	176	9	19.5:1
H II ₄ ♀ × Punjab Type 9 (α) ♂ (4)	117	114	3	38.0:1
" " (5)	176	166	10	16.6:1
TOTAL	804	758	46	16.5:1
Punjab Type 9 (β) ♀ × Punjab Type 16 ♂ (a)	603	567	36	15.7:1
" " (b)	567	539	28	19.2:1
Punjab Type 9 (β) ♀ × Moz. White ♂ (a)	705	661	44	15.0:1
" " (b)	441	403	38	10.6:1
Punjab Type 9 (β) ♀ × Punjab Type 25 ♂ (a)	1,020	961	59	16.3:1
" " (b)	591	543	48	11.1:1
Punjab Type 9 (γ) ♀ × Punjab Type ♂	987	907	80	11.3:1
TOTAL of all crosses with Type 9 ...	5,718	5,339	379	14.08:1
Expectation	...	5,360.6	357.4	

TABLE XI.

The F₂ generation of crosses between a three factor red and white parents.

Cross.	No. of Plants.	GRAIN COLOUR.		Ratio.
		Red.	White.	
American Club ♀ × Pusa 4 ♂ (1)	155	152	3	50.7 : 1
" " (2)	176	170	6	28.3 : 1
" " (3)	195	190	5	38.0 : 1
" " (4)	124	122	2	61.0 : 1
" " (5)	192	187	5	37.4 : 1
" " (6)	216	213	3	71.0 : 1
TOTAL	1,058	1,034	24	43.1 : 1
Expectation		1,041.5	16.5	
American Club ♀ × Pusa 6 ♂ (1)	132	131	1	131.0 : 1
" " (2)	211	208	3	69.3 : 1
" " (3)	178	175	3	58.3 : 1
" " (4)	97	96	1	96.0 : 1
" " (5)	180	179	1	179.0 : 1
" " (6)	201	199	2	99.5 : 1
TOTAL	999	988	11	89.8 : 1
Expectation		983.5	15.5	
American Club ♀ × Pusa 22 ♂ (1)	122	117	5	23.4 : 1
" " (2)	165	163	2	81.5 : 1
" " (3)	192	190	2	95.0 : 1
" " (4)	75	69	6	11.5 : 1
" " (5)	147	147	0	147.0 : 0
" " (6)	176	174	2	87.0 : 1
TOTAL	877	860	17	50.6 : 1
Expectation		863.2	13.7	
TOTAL of all crosses with American Club	2,934	2,882	52	55.4 : 1
Expectation		2,888.2	45.8	

It will be seen that three distinct kinds of splitting were obtained and that the numbers point to the 3 : 1, 15 : 1 and 63 : 1 ratios. The 15 : 1 ratio was obtained in all the crosses in which Punjab Type 9 was one of the parents thereby indicating the existence of two red factors in the grain colour. The 63 : 1 ratio was observed in all

the crosses in which a pure line of American Club was one of the parents. It will be observed in the figures relating to these ratios that eighteen plants belonging to three crosses were grown in the F_2 , that the mean ratio of red to white was 56.4 to 1, and the total number of plants involved was 2,934. Taking the progeny of the F_1 plants separately it will be seen the ratios varied between 11.5 to 1 and 179 : 1, while in one culture all the 147 plants had red grain. The greatest number of plants in any one culture was 216 and the smallest number 75. Compared with these the following figures obtained by Nilsson-Ehle in a similar case are of interest:—

0315 white × Swedish felted red	(a)	F_2	78	red	0	white.
" " "	(b)	"	30	"	0	"
" " "	(c)	"	49	"	0	"
" " "	(d)	"	31	"	0	"
" " "	(e)	"	86	"	0	"
" " "	(f)	"	110	"	0	"

Investigation of the third generation showed that in reality three red factors were present in the red parent and the F_2 should have given a ratio of 63 : 1 instead of all red plants. The Pusa numbers are undoubtedly better than those quoted from Svalöf, nevertheless it was a mistake to grow only some 200 or so individuals from each F_1 plant. It would have been better to have made a corresponding reduction in the total number of F_1 parents.

In the case of some of the crosses with Punjab Type 9, which gave the 15 : 1 ratio in the F_2 , the cultures have been carried on to the third generation and the two red factors isolated. (The crosses in which the 3 : 1 and 63 : 1 ratios were obtained are now being grown in the third generation.) On examining the grain of a large number of the F_2 plants which exhibited the 15 : 1 ratio it was observed that they formed a series and that those with red grain varied in colour from a very light yellowish red to tones as dark as the parent. A similar state of affairs to that observed in the felted chaff of these crosses was therefore obtained, but with the difference that no morphological characters corresponding to the two sets of hairs were present to assist in the unravelling of the series.

A large number of F_2 plants, representative of all the various tones of red in the series, were then sown singly seed by seed and the

resulting harvest examined. These plants gave rise in the F_3 to the following classes :—

1. Cultures in which plants with red and white grain occurred in the ratio 15 : 1.
2. Cultures in which plants with red and white grain occurred in the ratio 3 : 1.
3. Cultures in which all the plants had red grain, the red colour being uniform.
4. Cultures in which all the plants were red grained, but the red colour was not uniform in tone.
5. Cultures in which all the plants were white grained.

The presence of two independent red factors, indicated by the ratio obtained in the F_2 , was therefore confirmed by the behaviour of these plants in the F_3 .

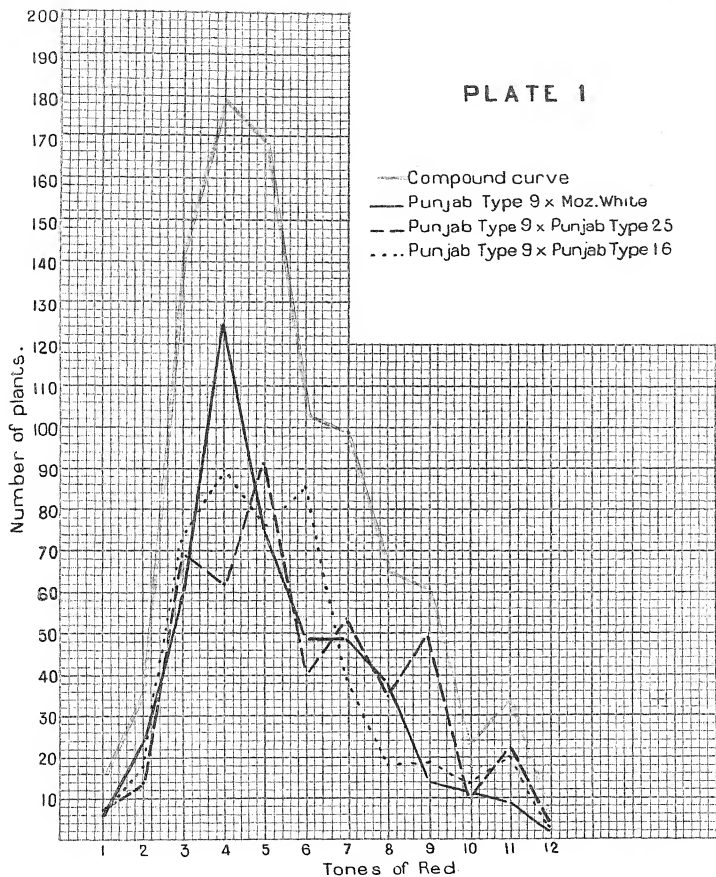
Representing the two red factors by R_1 and R_2 we therefore get the following scheme :—

$$\begin{array}{rcl}
 \text{Parents} & R_1 R_2 \times r_1 r_2 & \\
 & \underbrace{\hspace{1.5cm}} & \\
 & | & \\
 F_1 & R_1 r_1 R_2 r_2 & \\
 & | & \\
 F_2 & R_1 R_1 R_2 R_2 + r_1 r_1 r_2 r_2 + R_1 R_1 r_2 r_2 + r_1 r_1 R_2 R_2 + 2 R_1 r_1 R_2 R_2 & \\
 & + 2 R_1 R_1 R_2 r_2 + 2 R_1 r_1 r_2 r_2 + 2 r_1 r_1 R_2 r_2 + 4 R_1 r_1 R_2 r_2 &
 \end{array}$$

The appearance and mode of inheritance in these 9 classes are given in the following table :—

	Appearance of grain in F_2 .	Grain colour in F_3 .
$R_1 R_1 R_2 R_2$	red	red, uniform in tone.
$r_1 r_1 r_2 r_2$	white	white, uniform.
$R_1 R_1 r_2 r_2$	red	red, uniform in tone.
$r_1 r_1 R_2 R_2$	red	red, uniform in tone.
$R_1 r_1 R_2 R_2$	red	all red but not uniform.
$R_2 r_1 R_2 R_2$	red	all red but not uniform.
$R_2 r_1 r_2 r_2$	red	red and white in the ratio of 3 : 1.
$r_1 r_1 R_2 r_2$	red	red and white in the ratio of 3 : 1.
$R_2 r_1 R_2 r_2$	red	red and white in the ratio of 15 : 1.

PLATE 1



THE DISTRIBUTION OF RED TONES IN THE
SECOND GENERATION

As mentioned above the fact that the two red factors both produce a red colour makes the identification of these various classes impossible. Nevertheless it was found possible to isolate the two factors. In order to assist in the analysis of the F_2 it was decided to form a set of arbitrary standards by selecting all the tones of red occurring in this generation and to assign all the plants to classes represented by these standards. Twelve definite shades of red were obtained which covered the whole range of tones. In appearance standards 1, 2, 3, were a very pale yellowish red passing by degrees to the full red of 8, 9, 10, 11 and 12. Although grain belonging to standard 1 can always be distinguished with certainty from white grain, nevertheless the colour is so faint that it is more yellow than red.

In classifying the F_2 generation the grain of the twelve standards was placed in watch glasses on white paper in a good north light and the grains of the remaining F_2 plants were matched singly and their respective places in the series determined.

The distribution of the F_2 plants as regards the tone of colour of the red grained individuals is given in the following table and again graphically in Plate I. The red curve is compounded of the three single curves given by three crosses.—

CROSS.	No. of plants.	RED STANDARDS.											
		1	2	3	4	5	6	7	8	9	10	11	12
Punjab Type 9												x	
F_1 of all crosses							x						
Punjab Type 9 x Punjab Type 16.	594	8	21	98	107	91	102	46	22	21	15	24	3
Punjab Type 9 x Moz. white.	705	9	34	86	178	109	70	70	54	20	15	13	3
Punjab Type 9 x Punjab Type 25.	954	13	27	137	120	184	78	105	68	96	20	42	6
TOTAL	2,253	30	82	321	405	364	250	221	144	137	50	79	12

It will be observed that in the graphic representation the twelve red tones have been assumed for the sake of convenience to differ from one another by an equal amount. This is probably not the case, but does not affect the argument. For all the crosses the numbers have been calculated for 500 plants in order to make the comparison of the curves easier.

The red grained parent, Punjab Type 9, was found to be identical with the eleventh standard and the F_1 in all cases was intermediate between standards 5 and 6. The bulk of the plants in the F_2 appear in the centre of the curve. The curves obtained from the individual crosses agree very fairly well with each other and with the compound curve, especially if the difficulty of matching these slightly varying shades of red be taken into account. All the observations on this question both in the F_2 and F_3 generations were carried out by the same observer in order to minimise the personal equation.

A large number of F_2 plants, representative of all the various tones of red in the series, were then sown singly seed by seed and the resulting harvest examined in the same manner as the F_2 . By this examination it was possible to identify the two shades of red representing the two factors R_1 and R_2 and to isolate wheats which, in all probability, contain each factor singly. It was found that the F_3 cultures derived from F_2 plants with grain belonging to the classes represented by standards 1 and 2 in all cases gave a ratio of 3 red grained to 1 white grained plant, the darkest red tone obtained being never higher than 5. The F_3 plants represented by standards 3 and 4 also gave the 3 : 1, but in each case some plants bred true to red and gave uniform progeny with pale red grains. A few plants giving the same pale red uniform progeny were also derived from standard 5, but generally speaking after standard 4 there is a distinct change in the nature of the F_3 cultures. Ratios of 15 red grained to 1 white, were obtained from standard 5 as well as some with 3 red to 1 white, but in this case the red tones ranged as high as standard 9. It is clear that in the region of the curve, in which standards 3 and 4 occurred, one of the red factors was to be found and this was represented by the pale constant reds obtained. The pale red F_2 plants represented by standards 1 and 2 which gave the 3 : 1 ratio must have been heterozygotes of the pale red and white gametes. In a similar manner many uniform red plants occurred at 9 and a few at standard 8. These were a full red and represented the other red factor. The plants in standard 5 which gave the 3 : 1 ratio with red tones up to standard 9 must have been heterozygotes of the dark red and white factors. The reds which were not uniform were met

with from standard 5 onwards, some ranged from 3 to 11, others from 9 to 12, and these must have been heterozygotes containing both the red factors. Thus the two red factors in the original parent have been isolated, the pale red factor corresponding to standard 4 and the darker red to standard 9. When both are present a dark red grain is obtained corresponding to standard 11, the tone of the original parent. The five sets of the heterozygotes are always intermediate in tone and with the four constant forms complete the series. The large preponderance of F_2 plants between standards 3 and 6 is easily explained by the occurrence at this point of the following forms— $R_1 r_1 R_2 r_2$, $R_1 R_1 r_2 r_2$, and $R_1 r_1 R_2 R_2$. It will be seen that secondary maxima occur at 9 and 11 corresponding to the pure dark red factor and the two red factors combined.

CHAFF COLOUR.

The investigations hitherto published on the inheritance of chaff colour are summed up by Tschernak in the 1910 edition of *Die Züchtung der land. Kulturpflanzen*.

In the five crosses we have made between red and white chaffed wheats, the results have been similar, and in all cases, after an intermediate F_1 generation, simple splitting in the ratio of 3 red to 1 white was obtained in the F_2 . The results of the examination of 4,722 plants belonging to these five crosses are given in the following table:—

TABLE XII.

The F_2 generation of crosses between red and white chaffed parents.

CROSS.		No. of plants.	CHAFF COLOUR.		Ratio.
			Red.	White.	
A 88 ♀ × Pusa 22 ♂	(1)	168	120	48	2.5 : 1
" " "	(2)	247	193	54	3.6 : 1
" " "	(3)	258	158	70	2.7 : 1
Pusa 22 ♀ × A 88	(4)	117	83	34	2.4 : 1
" " "	(5)	196	145	51	2.8 : 1
TOTAL		986	729	257	2.8 : 1
Expectation			739.5	241.5	

TABLE XII—continued.

The F_2 generation of crosses between red and white chaffed parents—continued.

CROSS.	No. of plants.	CHAFF COLOUR.		Ratio.
		Red.	White.	
B XI 77 ♀ × HI ₂ ♂ (1)	194	151	43	3.5: 1
" " (2)	227	175	52	3.4: 1
" " (3)	233	190	43	4.4: 1
HI ₂ ♀ × B XI ₂ ♂ (5)	253	189	64	2.9: 1
TOTAL	907	705	202	3.5: 1
Expectation		680.25	226.75	
American Club ♀ × Pusa 4 ♂ (1)	156	120	36	3.3: 1
" " (2)	177	136	41	3.3: 1
" " (3)	195	146	49	3.0: 1
" " (4)	124	95	29	3.3: 1
" " (5)	192	139	53	2.6: 1
" " (6)	216	164	52	3.1: 1
TOTAL	1,060	800	260	3.1: 1
Expectation		795	265	
Punjab Type 9 (a) ♀ × HI ₄ ♂ (1)	137	106	31	3.4: 1
" " (2)	189	139	50	2.2: 1
" " (3)	185	138	47	2.9: 1
HI ₄ ♀ × Punjab Type 9 (a) ♂ (4)	117	87	30	2.9: 1
" " (5)	176	134	42	3.2: 1
TOTAL	804	595	209	2.8: 1
Expectation		603	201	
Punjab Type 9 (γ) ♀ × Punjab Type 13 ♂	965	741	224	3.3:
Expectation		723.75	241.25	

IV. THE PRESENCE AND ABSENCE OF AWNS.

THE inheritance of awns in wheat has been investigated by several observers and the results obtained are to be found in von Tschermak's summary in *Die Züchtung*, which has been referred to under chaff colour. Biffen, Wilson, Schribaux and von Tschermak found that the beardless condition was dominant and that the F_2 generation was composed of bearded and beardless plants in the simple ratio 3 : 1. In some cases the ratio fully bearded to half bearded and beardless in this generation was 1 : 2 : 1. Nilsson-Ehle found however that the beardless condition was not absolutely dominant. Von Tschermak observed in two cases in crossing a bearded and beardless wheat that the bearded condition was entirely lost, while Rimpau and Spielmann on crossing two beardless wheats obtained in the F_2 a few bearded forms which bred true. Saunders¹ in 1906 combated the statement that the first generation between a beardless and a bearded wheat is always beardless, and maintained that the amount of bearding in the F_1 varies with the wheats used. In the F_2 a series of forms, which defied classification, was obtained. Apparently the subject was not further investigated as no details appear to have been published since.

In our investigations at Pusa with Indian wheats the inheritance of the fully awned character has not been simple. In crosses between bearded and beardless wheats two very distinct phenomena have been observed. In one series, the plants in the F_1 were distinctly intermediate and were half bearded, while in the other only very short tips to the glumes occurred. These differences in the F_1 were correlated with differences in the beardless parents. In the first case these had short tips to the

¹ Saunders, *Report of the Third International Conference 1906 on Genetics*, London, 1907, p. 370.

glumes, while in the second they were absolutely beardless. In the following paragraphs these two classes are dealt with separately.

The half bearded F_1 generation occurred in four crosses between bearded and beardless wheats and the results (Plate II) differ entirely from those illustrated by Biffen and Wilson in Vol. 1 of the *Journal of Agricultural Science*. The four crosses with an intermediate F_1 all broke up in the F_2 into fully bearded, half bearded like the F_1 and plants with tipped glumes in the ratio 1 : 2 : 1. There was no difficulty in distinguishing these classes which were very distinct the one from the other. The details relating to the 2,836 F_2 plants of these four crosses are given in the following table:—

TABLE XIII.

The F_2 generation of crosses between bearded and tipped parents.

Cross.	No. of Plants.	F_2 GENERATION.			Ratio.
		Bearded.	Inter-mediate.	Tipped.	
BXI 77 ♀ × HI ₂ ♂ (1)	194	47	105	42	1.1 : 2.5 : 1
" " (2)	227	67	113	47	1.4 : 2.4 : 1
" " (3)	233	52	116	65	.8 : 1.8 : 1
HI ₂ ♀ × BXI 77 ♂ (5)	253	62	123	68	.9 : 1.8 : 1
TOTAL	907	228	437	222	1 : 2.1 : 1
Expectation		226.75	453.5	226.75	
Punjab Type 9 (a) ♀ × HII ₄ ♂ (1)	137	35	71	31	1.1 : 2.3 : 1
" " (2)	190	54	89	47	1.1 : 1.9 : 1
" " (3)	185	46	87	52	.9 : 1.7 : 1
" " (4)	117	32	55	30	1.1 : 1.8 : 1
HII ₄ ♀ × Punjab Type 9 (a) ♂ (5)	175	45	89	38	1.3 : 2.3 : 1
TOTAL	804	215	391	198	1.1 : 2.0 : 1
Expectation		201	403	201	
Punjab Type 9 ♀ × Punjab Type 25 ♂	1,022	244	528	250	1 : 2.1 : 1
Expectation		255.5	511	255.5	
American Club ♀ × Pusa 6 ♂ (1)	133	42	56	35	1.2 : 1.6 : 1
" " (2)	215	62	98	55	1.1 : 1.8 : 1
" " (3)	178	58	81	39	1.5 : 2.1 : 1
" " (4)	97	24	49	24	1.0 : 2.0 : 1
" " (5)	179	47	85	47	1.0 : 1.8 : 1
" " (6)	201	60	90	51	1.2 : 1.8 : 1
TOTAL	1,003	298	469	251	1.2 : 1.8 : 1
Expectation		250.75	501.5	250.75	



Punjab Type 9.



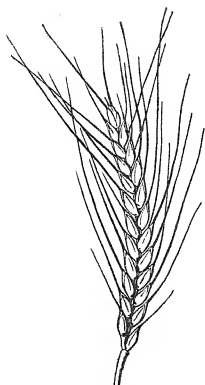
6



Parents.

H II,

First
Generation.



Fully bearded.



Intermediate.



Tipped.

Second
Generation.

THE RESULT OF CROSSING BEARDED AND TIPPED WHEATS.

In one of these crosses (Punjab Type 9 \times Punjab Type 25), a large number of the three kinds of F_2 plants were grown and the F_3 generation examined. All the fully bearded plants and those with tips bred true, while the half bearded plants again split up into fully bearded, half bearded and tipped plants in the ratio 1 : 2 : 1. The details relating to thirty-one of these cultures are given in the following table:—

TABLE XIV.

The F_3 generation of a cross between bearded and tipped parents.

Plant No.	Bearding in F_2 parent.	No. of plants in F_2 .	BEARDING IN THE F_3 GENERATION.			Ratio.
			Fully bearded.	Intermediate.	Tipped.	
10	Fully bearded	77	77	
31	do.	90	90	
218	do.	77	77	
233	do.	71	71	
319	do.	81	81	
459	do.	74	74	
470	do.	122	122	
499	do.	87	87	
611	do.	139	139	
649	do.	122	122	
924	do.	132	132	
65	Tipped	75	75	
138	do.	99	99	
509	do.	102	102	
566	do.	115	115	
582	do.	91	91	
787	do.	96	96	
853	do.	94	94	
940	do.	88	88	
1004	do.	113	113	
1026	do.	94	94	
1	Intermediate	60	16	26	18	
4	do.	49	15	27	7	
30	do.	93	21	49	23	
75	do.	86	19	41	26	
79	do.	62	14	27	21	
184	do.	63	18	33	12	
317	do.	83	17	43	23	
368	do.	70	15	31	24	
401	do.	90	29	38	23	
468	do.	77	19	42	16	
481	do.	66	15	34	17	
507	do.	70	17	35	18	
623	do.	51	12	28	11	
780 \times	do.	62	13	27	17	
803	do.	62	12	39	15	
818	do.	66	17	46	17	
863	do.	80	16	23	16	
911	do.	55	20	46	23	
973	do.	89	20	44	16	
990	do.	80	20	44	15	
		87	14	38		
TOTAL		1419	344	717	358	
Expectation			354.75	709.5	354.75	.96 : 2 : 1

The crosses between bearded and beardless plants in which the F_1 generation was apparently beardless will now be considered.

This was observed in the following pure line crosses in which the bearded parent is placed first :—

1. American Club \times Pusa 4.

2. Pusa 22 \times A 88.

In both the beardless parents Pusa 4 and A 88 there was an entire absence of tips, and these parents could be described as absolutely beardless. In the second generation of these crosses a series of forms was produced which we have classed with difficulty as fully bearded, almost fully bearded, half bearded, forms with tips of varying length and beardless (Plate III). If all the variously bearded and tipped plants are classed as bearded, which would seem to be the most logical method, the ratio bearded to beardless obtained was 15 : 1 and the presence of two factors is indicated. Representatives of the various classes are now being grown in the F_2 and the full explanation, as well as the complete study of the F_2 , will be dealt with next spring as soon as the plants are ripe. The details relating to the F_2 generation of these crosses are given in the following table :—

TABLE XV.

The F_2 generation of crosses between bearded and quite beardless parents.

CROSS.	No. of plants.	BEARDING IN THE F_2 GENERATION.				Beardless.
		Fully bearded.	Almost fully bearded.	Half bearded.	Tips of varying length.	
American Club $\varnothing \times$ Pusa 4 σ (1)	156	8	11	24	105	8
" " " (2)	176	12	11	27	113	13
" " " (3)	197	10	12	24	138	13
" " " (4)	124	6	9	14	84	11
" " " (5)	192	11	13	24	132	12
" " " (6)	216	11	14	22	155	14
TOTAL	1061	58	70	135	727	71
Ratio	...	8	1	1.9	10.2	1
A 88 $\varnothing \times$ Pusa 22 σ (1)	168	12	11	19	117	9
" " " (2)	247	18	11	28	173	17
" " " (3)	258	16	20	31	174	17
Pusa 22 $\varnothing \times$ A 88 σ (4)	117	5	11	17	76	8
" " " (5)	196	10	12	29	133	12
TOTAL	986	61	65	124	673	63
Ratio		1	1	2	10.7	1



Pusa 22.



A 88.

Parents.



First
Generation.



Fully bearded.



Nearly fully
bearded.



Half
bearded.



Long
tips.



Short
tips.



Beardless.

Second
Generation.

THE RESULT OF CROSSING BEARDED AND BEARDLESS WHEATS.

The distinction between the above two kinds of inheritance in bearding is of great interest. In one case the simple Mendelian scheme is followed, but there is no question of dominance in the F_1 , which is clearly intermediate. In all these cases however one of the parents is only beardless in the ordinary sense of the term, short tips to the glumes being present. In the other case the F_1 is almost beardless while in the F_2 the 15 : 1 ratio is obtained when all plants with beards, whatever may be their length, are separated from the absolutely beardless ones. Here, however, one of the parents is absolutely beardless.

In the following crosses the same bearded parent (American Club) occurs in both classes—

- (1) American Club (bearded) \times Pusa 6 (beardless) with the 1 : 2 : 1 ratio in the F_2 .
- (2) American Club (bearded) \times Pusa 4 (beardless) gave the 15 : 1 ratio in the F_2 .

Pusa 6 has short tips to the awns while Pusa 4 is absolutely beardless. This appears to indicate that in American Club two factors are present, a long and short factor, while both are absent in Pusa 4 and only the short factor is present in Pusa 6.

Before discussing the possible explanations of these phenomena the results of the other crosses made with bearded parents must be stated. In crosses between plants with slight tips to the awns and absolutely beardless parents the F_1 generation was intermediate and the F_2 gave fully tipped, intermediate and absolutely beardless plants in the ratio 1 : 2 : 1 and no difficulty was experienced in making the separation. Crosses between fully bearded parents gave similar offspring in the F_1 and again in the F_2 . In crosses between tipped parents the F_1 and F_2 generations were alike and resembled the parents in having all the awns with tips of similar length. These results are given in the following table :—

TABLE XVI.

The F₂ generation of crosses between tipped and beardless parents.

Cross.		No. of plants.	F ₂ GENERATION.			Ratio.
			Fully Tipped.	Intermediate.	Beardless.	
Biffen's hybrid ♀ × Pusa 4 ♂	(2)	178	42	94	42	
"	(5)	221	49	129	43	
"	(6)	197	39	101	57	
TOTAL		596	130	324	142	9 : 23 : 1
			454			
Biffen's hybrid ♀ × Pusa 4 ♂	(1)	158		113	45	
"	(3)	110		81	29	
"	(4)	115		91	24	
TOTAL of all 6 plants		979		789	240	3:07:1
Expectation				734.25	244.75	

TABLE XVII.

The F₂ generation of crosses between bearded parents.

Cross.		No. of plants.	Nature of Progeny in F ₂
American Club ♀ × Pusa 22 ♂	(1)	122	All fully bearded.
"	(2)	165	" " "
"	(3)	192	" " "
"	(4)	75	" " "
"	(5)	147	" " "
"	(6)	176	" " "
TOTAL		877	" " "
American Club ♀ × Pusa 23		137	All fully bearded.
Punjab Type 9 ♀ × Punjab Type 16 ♂		1188	" " "
Punjab Type 9 ♀ × Mez. White ♂		1150	" " "
Punjab Type 9 ♀ × Punjab Type 13 ♂		965	" " "

TABLE XVIII.

The F₂ generation of crosses between tipped parents.

Cross.	No. of plants.	Beardless.	Tipped.
Biffen's hybrid ♀ × Pusa 6 ♂ (3)	179	179
" " (6)	163	163
TOTAL	342		342

The complete explanation of these ratios in the F₂ and the isolation of the two factors concerned in the 15 : 1 ratio can only be arrived at after the F₂ generation of this latter cross has been grown and the results examined in detail. The simplest explanation appears to be that in the bearded parent two factors are present, one capable of producing short awns or tips only, the other resulting in fully bearded plants, while in the tipped parents the short factor only is present. On the presence and absence hypothesis, the results of the various crosses would be represented as follows: Assuming the fully bearded parent contains two factors B and T (B representing the long and T the short factor), the tipped plants contain only one factor T, while the absolutely beardless plants contain neither, then—

- (1) Fully bearded wheats would be represented by B B T T.
- (2) Plants with tips only would be represented by b b T T.
- (3) Absolutely beardless plants would be represented by b b t t.

The various crosses described above would then be represented as follows :—

1. Fully bearded (BBTT) × tipped (bbTT) would give in the F₁ BbTT and in the F₂ 1 BBTT + 2BbTT + 1 bbTT.
2. Fully bearded (BBTT) × beardless (bbtt) would give BbTt in the F₁ and the F₂ would be represented by the formula :—
BBTT + 4 BbTt + 2 BbTT + 2 BBtt + bbTT + 2 bbTt + BBtt + 2 Bbtt + bbtt.
3. Tipped (bbTT) × beardless (bbtt) would give in the F₁ bbTt and in the F₂ bbTT + 2 bbTt + bbtt.

4. Bearded (BBTT) \times Bearded (BBTT) would give BBTT in both the F_1 , and the F_2 .
5. Tipped (bbTT) \times Tipped (bbTT) would give the same bbTT in both the F_1 and the F_2 .

Whether the above explanation, in which the bearded parent contains two factors, will hold or not will be proved by the study of the F_2 generation. Another explanation has been suggested by von Tschermak, namely, the presence of a restraining factor in the beardless parent. This, however, does not seem to fit the facts so easily as that put forward by ourselves, and in this connection it should be noted that the sharp distinction here drawn between absolutely beardless and so-called beardless plants with short tips does not seem to have been made before. It appears that tipped plants have been regarded as beardless in most of the European work and that crosses with really beardless plants have not been made. In our Indian collection most of the "beardless" plants are tipped and absolutely beardless plants, which are the best analysers for the study of the bearded character, are rare.

COLOUR OF THE AWNS.

In many of the Indian wheats the awns are black, whenever the wheats are fully developed. If, however, premature ripening takes place, this colour is not developed. In one cross between a black and a white awned wheat the inheritance of black awns in the F_1 was determined. The black awns prevailed in the F_1 and in the F_2 the ratio of black awns to white was 3.45 to 1, the actual numbers being 466 black and 135 white. It was not possible to separate the fully black awned plants from the intermediates, but the occurrence of these two classes was evident. The parents used were Punjab Type 9 (black awned), and Punjab Type 16 (white awned).

V. OTHER CHARACTERS.

In addition to the investigations on the inheritance of felted chaff, red grain colour and the bearding of wheat, some other characters have been studied, three of which are referred to briefly in this section. The complete investigations on these points will be dealt with in later papers. The characters in question are the consistency of the grain, the shattering of the ear and standing power.

CONSISTENCY OF THE GRAIN.

The results of our investigations on the influence of the environment on the consistency of wheat are now being published. It has been found that wheats can be divided into three groups as regards this matter. In the majority of Indian wheats, whether soft or translucent, this character is greatly influenced by the environment, and soft wheats are easily changed to translucent and *vice versa*. A few translucent wheats, however, have a decided tendency to remain translucent under all conditions, while some soft wheats have so far remained soft no matter how or where they are grown. In one of the crosses at Pusa one of these constant soft white wheats was crossed with a strong translucent amber wheat and the segregation was so clear in the F_2 that counts were made. The F_1 was intermediate and the F_2 split sharply into soft, semi-translucent and translucent in the ratio 1 : 2 : 1. It will be seen from the following table that the results obtained are practically identical with expectation:—

TABLE XIX.

The F₂ generation of a cross between translucent and soft grained parents.

CROSS.	No. of plants.	CONSISTENCY.			Ratio.
		Soft.	Semi-translucent.	Translucent.	
A 88 ♀ × Pusa 22 ♂ (1)	167	39	82	46	1 : 2.1 : 1.2
" " (2)	247	58	132	57	1 : 2.3 : 1
" " (3)	256	67	125	64	1 : 1.9 :
Pusa 22 ♀ × A 88 ♂ (5)	196	43	108	45	1 : 2.5 : 1
TOTAL ..	866	207	447	212	1 : 2.1 : 1
Expectation		210.5	433	216.5	

TABLE XX.

The Nitrogen content in the F₂ generation of a cross between translucent and soft grained parents.

		Consistency.	Nitrogen percentage.
A 88		Soft	2.21
Pusa 22		Translucent	2.51
A 88 × Pusa 22 F ₂ 1		Soft	2.32
" " 2		"	2.42
" " 3		"	2.29
" " 4		"	2.43
" " 5		"	2.32
" " 6		"	2.07
A 88 × Pusa 22 F ₂ 7		Semi-translucent	2.31
" " 8		"	2.32
" " 9		"	2.57
" " 10		"	2.35
" " 11		"	2.53
" " 12		"	2.30
A 88 × Pusa 22 F ₂ 13		Translucent	2.50
" " 14		"	2.78
" " 15		"	2.36
" " 16		"	2.67
" " 17		"	2.35
" " 18		"	2.43

A similar sharp segregation was observed in some of the crosses made at Pusa in 1906, but the figures were not published as one of the parents used was not a pure line.

Some nitrogen determinations¹ were made of the various classes in the F_2 , and these are given in the table above. It will be seen that both parents are high in nitrogen, and that in the second generation there is no connection between translucency and high nitrogen content, but that both these characters are inherited independently. Plants with low and high nitrogen occur in all the classes. It is proposed to study this cross in detail from the point of view of the inheritance of strength and free-milling qualities.

SHATTERING OF THE EAR.

In India, one of the important characters of wheat from the economic standpoint is the power of holding the grain in the ear when the wheat is ripe. At harvest time the temperature is high, the air exceedingly dry and high winds are common. Moreover, it is the custom to allow the wheat to become dead ripe before it is cut, and owing to scarcity of labour at harvest time it often remains standing in the fields long after it is ripe. Under these circumstances many wheats drop their grain and a great loss of wheat results. In the improvement of Indian wheat any tendency to shed grain is a fatal defect in a new wheat, and several otherwise excellent types have had to be discarded by us on this account. In one of our crosses a distinct segregation in the F_2 was noticed as regards the shattering of the ear, and the 15 : 1 ratio was perfectly distinct. Two factors must therefore have been present, one of which it is likely to be found in Pusa 6 as this line has a tendency to shed its grain, but the shattering of many plants in the F_2 was much greater than in Pusa 6.

The details relating to the F_2 of the cross in question are given in the following table :—

Name of cross.	No. of plants.	Non- shattering.	Shattering.	Ratio.
American Club \times Pusa 6 (2)	215	12	203	1 : 16.9

¹ We are indebted to Dr. Leather, Imperial Agricultural Chemist, for these nitrogen determinations.

STANDING POWER.

Perhaps the most important character in the improvement of Indian wheat at the present time is standing power. Now that it has been shown to be possible in the plains of India to raise crops exceeding forty bushels to the acre, it is clear that good standing power is essential to carry this weight of grain and for the crop to remain erect after the storms of wind and rain which often occur after the plants are in ear. Most of the Indian wheats have very weak straw, only suitable for carrying crops of about twenty bushels to the acre. In improving wheat production, therefore, both as regards methods of cultivation and quality, great attention has to be paid to standing power. Standing power appears to be due to at least two factors, first to strong straw, which is generally associated with very erect ears, and, secondly, to what may be called power to form a strong root system. In one of our crosses a parent with strong straw but inferior rooting power (A 88) was crossed with a wheat with weak straw but which rooted well (Pusa 22) and the F_2 plants were examined in detail. All combinations of these factors were evident in the F_2 —well-rooted wheats with strong straw, well-rooted wheats with weak straw, wheats with strong straw and inferior rooting power as well as some which had neither good straw nor good rooting power. Careful records were kept of individual plants, but in a character like this it is not possible to secure accurate counts. This evidence of distinct segregation renders it likely, however, that it will be an easy matter to secure wheats with strong straw and good rooting power and to increase the standing power of the Indian wheats very considerably.

VI. SUMMARY AND CONCLUSIONS.

The results obtained in these investigations may be summed up as follows :—

1. The hairs on the chaff of felted wheats vary considerably in length and density and one or more kinds of hairs may be present in the same wheat. In all the hybridization experiments carried out so far, each kind of hair has proved to be due to a single factor, and these factors are inherited independently of one another. The number of kinds of hairs and consequently of gametic factors involved in the felting of wheats is probably large. All of the Indian macaroni wheats examined up to the present and also all densely felted wheats have two kinds of hairs.

2. The red colour of the grain of wheat may be due to the presence of one, two or three factors which are inherited independently of each other. Each is capable of producing a red colouration, but the shade of red thus produced varies. It is possible to isolate from a cross between a red wheat with a multiple grain colour and a white wheat, wheats possessing grains of intermediate shades of red which bred true at once. The effect of the two red factors in Punjab Type 9 is cumulative, and the colour of the grain of this wheat is deeper than that produced by either of the two factors singly. Only one factor was found to occur in all the crosses between red and white chaffed wheats which after an intermediate F_1 gave the usual 1 : 2 : 1 ratio in the F_2 .

3. The existence of at least two factors in the bearding of wheats has been demonstrated, and it has been shown that a sharp distinction must be drawn in the future between wheats which are absolutely beardless, and those with very short awns or tips. The usual practice of classing all wheats with awns not longer than one quarter of an inch or so as beardless is misleading.

4. The black colour of the awns prevailed in the cross Punjab Type 9 \times Mozaffarnagar white and segregation in the F_2 of three black to one white awned plant was obtained.

5. On crossing a constant soft wheat with a translucent one, distinct segregation into soft, intermediate and translucent wheats was obtained in the Mendelian ratio of 1 : 2 : 1. This segregation was independent of the nitrogen percentage.

6. In the shattering of the ear two factors appear to be involved and distinct segregation with intensification was observed in the F_2 .

7. Segregation was also observed in a cross between a weak strawed well rooted wheat and a strong strawed wheat with inferior rooting capacity. All possible combinations were found, and the prospect of obtaining a well rooted strong strawed wheat in the future seems favourable.

8. In none of the crosses was there any indication of dominance in the first generation, which in all cases proved to be intermediate.

From the general standpoint of the improvement of crops the more modern investigations on hybridization are not without interest. In concluding this account an attempt will be made to indicate the bearing of these investigations on the wider aspects of plant breeding work conducted with the object of obtaining better staples than those now cultivated. At the present time in many countries there is a considerable amount of state-aided effort devoted to the advancement of agriculture, and one of the chief activities of the various Agricultural Departments is concerned with plant breeding and selection.

1. THE PRESERVATION OF PURE LINES.

The most modern investigations on hybridization confirm the necessity of starting all plant breeding work from pure lines, the

characters of which have been studied adequately. In the present state of knowledge, this is undoubtedly the best material available for the purpose and, consequently, the preservation of the pure lines for the benefit of future workers becomes an important matter. This is particularly so in countries like India where reliable seed merchants do not exist, and where it is practically impossible to procure varieties of crops even botanically pure. Before hybridization work can be begun in India, it is necessary to make surveys of the particular crops studied and to separate them not only into botanical varieties and finally into pure lines but also to determine with precision all the characters of the pure lines themselves. All this preliminary work necessarily involves the expenditure of considerable time and money. As hybridization work proceeds more and more, pure lines of known gametic constitution will become, as it were, accumulated at the various stations, and the proper preservation of this material is an important matter, and becomes a valuable asset of the Agricultural Department concerned. It is important that it should not be lost and that it should be readily available for future workers in a manner somewhat similar to the way in which, at research centres, libraries of books are handed down to posterity.

The preservation and exchange of pure lines, the gametic constitution of which has been proved, might easily become of very general importance. There is little doubt that hybridization work will, in the future, become in each country more and more restricted to a few centres, and it is very probable that the material of one country may be of use to the workers at centres in other countries. Thus there may very easily be useful exchanges of pure lines in Northern Europe and again in North America. The whole subject of the best means of preserving pure lines of economic plants of known gametic constitution is one which might well be taken up by the next International Conference on Genetics or by the International Association of Botanists.

II. PLANT BREEDING STATIONS.

The complexity of such apparently simple characters in wheat

as those dealt with in the present paper leaves little doubt that the number of factors involved in characters of economic importance such as strength of flour, rust resistance and standing power are equally great. This in turn renders the isolation of new kinds which bred true in all respects, from the progeny of a cross, a matter of greater labour and longer time than was at one period suspected. In addition many of these characters are subject to the influence of environment, so that both the study of inheritance and the work of breeding improved varieties becomes increasingly difficult. If progress is to be made in the elucidation of the laws of inheritance on the one hand and in the production of improved crops on the other, it seems difficult to resist the conclusion that there can be little or no progress in either direction unless the work is organised in such a manner that it is restricted to a few centres adequately equipped. The publication of the early researches on inheritance, which followed closely on the rediscovery of Mendel's law, undoubtedly stimulated a large amount of hybridization work at the Agricultural Experiment Stations and was the means of raising expectations that the improvement of crops was a simple matter and might be accomplished in a very short period. As a consequence, plant breeding was started at stations as an addition to an already overloaded programme and the result has been to flood the literature with a mass of superficial results of no permanent value. Whenever plant breeding has been done with thoroughness and on a sufficiently large scale, it has invariably been found that the inheritance of characters is by no means such a simple matter as was first supposed, and the investigations conducted at such centres as Svalöf explain why it is that the numerous attempts at plant improvement made at the Agricultural Experiment Stations have not led to any very striking results. There is no doubt that plant breeding work is useless unless it is carried out on a large scale and with great thoroughness. This in turn can only be done effectively at Experiment Stations at which this work is made the chief item of the programme. It would be better, therefore, for each country to maintain a few good plant improvement stations than to carry on superficial investigations at many centres.

III. THE BASIS OF SELECTION.

The results obtained in the present paper have a considerable bearing on selection. It has been shown in wheat that characters, which appear at first sight to be simple, are in reality made up of several factors, each inherited independently of one another. The total number of factors in this crop will no doubt be found to be considerable. Natural cross-fertilization has been shown to be much commoner than was at one time suspected, and this supplies the means by which these factors can combine together to form a very large number of wheats, differing from each other by small amounts. The known complexity of botanical varieties in wheat is at once explained by the interplay between the numerous factors rendered possible by natural crossing. Consequently, the wheats of any region and especially those of a country like India, in which agriculture has been practised from time immemorial, supply material, which may well turn out to be a veritable gold mine, for the exercise of systematic schemes of selection. The careful comparison of the offspring of single plants may yield results of great value to the country. A similar state of affairs appears, from our observations in India, to obtain in several other crops in which self-fertilization is the rule. The comparison of the pure lines of these self-fertilized crops offers a line of work which may prove to be of the very greatest importance in agriculture and is moreover much simpler than hybridization investigations. The only difficulties involved are those relating to the interpretation of the results of the field trials in deciding whether or not an improvement has really been obtained.

These results also concern the question of the improvement of plants in which crossing is common. Here there is little doubt also that numerous factors are involved. These, however, have crossed so much among themselves that there has been no opportunity for the production of pure lines, so that the crops are a network of freely intercrossing forms. A large amount of systematic selection, extending over a considerable period, is therefore necessary before material, in any sense approximating to pure lines, can be obtained. While, therefore, the question of selection in self-fertilized crops is

seen to be established more firmly as a result of recent work on hybridization, the difficulties in applying this process with success to cross-fertilized crops appear to be very much greater. Further, the question of the maintenance of the vigour of these latter crops, when grown in pure culture and when crossing is prevented, is a subject which, up to the present, has not received a very large amount of attention.

QUETTA,

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THE INFLUENCE OF THE ENVIRONMENT ON
THE MILLING AND BAKING QUALITIES OF
WHEAT IN INDIA.

No. 2. THE EXPERIMENTS OF 1909-10 AND 1910-11.

BY

ALBERT HOWARD, M.A., F.L.S.
Imperial Economic Botanist.

H. M. LEAKE, M.A., F.L.S.
*Economic Botanist to the Government
of the United Provinces.*

AND

GABRIELLE L. C. HOWARD, M.A.
*Personal Assistant to the Imperial Economic Botanist,
Associate and Former Fellow of Newnham College, Cambridge.*



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PREFACE.

THE experiments described in the present paper are a continuation of those dealt with in a previous communication (*Mem. Dept. Agr. in India, Bot. Ser., No. 4, Vol. III, 1910*).

We desire to take this opportunity of expressing our indebtedness to several officers of the Indian Agricultural Department for their valuable co-operation in the conduct of this work. For facilities at Dumraon and Bankipore we are indebted to Mr. F. Smith, Deputy Director of Agriculture, Bengal. Dr. Parr and Mr. B. C. Burt, the Deputy Directors of Agriculture of the United Provinces, have assisted us at Aligarh and Orai respectively. At Meerut, Babu Jagannath Pershad gave us all facilities on his farm. Khan Bahadur Mohamad Hadi has kindly placed the resources of the Partabgarh Experiment Station at our disposal, while at Lyallpur, Mr. W. Roberts, Professor of Agriculture, has been good enough to assist in the work. For facilities at Hoshangabad in the Narbada Valley we are indebted to Mr. G. Evans. At Raipur, in the Chattisgarh Division of the Central Provinces, Mr. D. Clouston, Deputy Director of Agriculture, has given us very valuable help.

For the very large number of nitrogen determinations involved in the work we are indebted to Dr. J. W. Leather, Imperial Agricultural Chemist.

In the milling and baking aspect of the subject we have been fortunate enough to secure the invaluable assistance of Mr. A. E. Humphries, formerly President of the National Association of British and Irish Millers and a well-known authority on these questions.

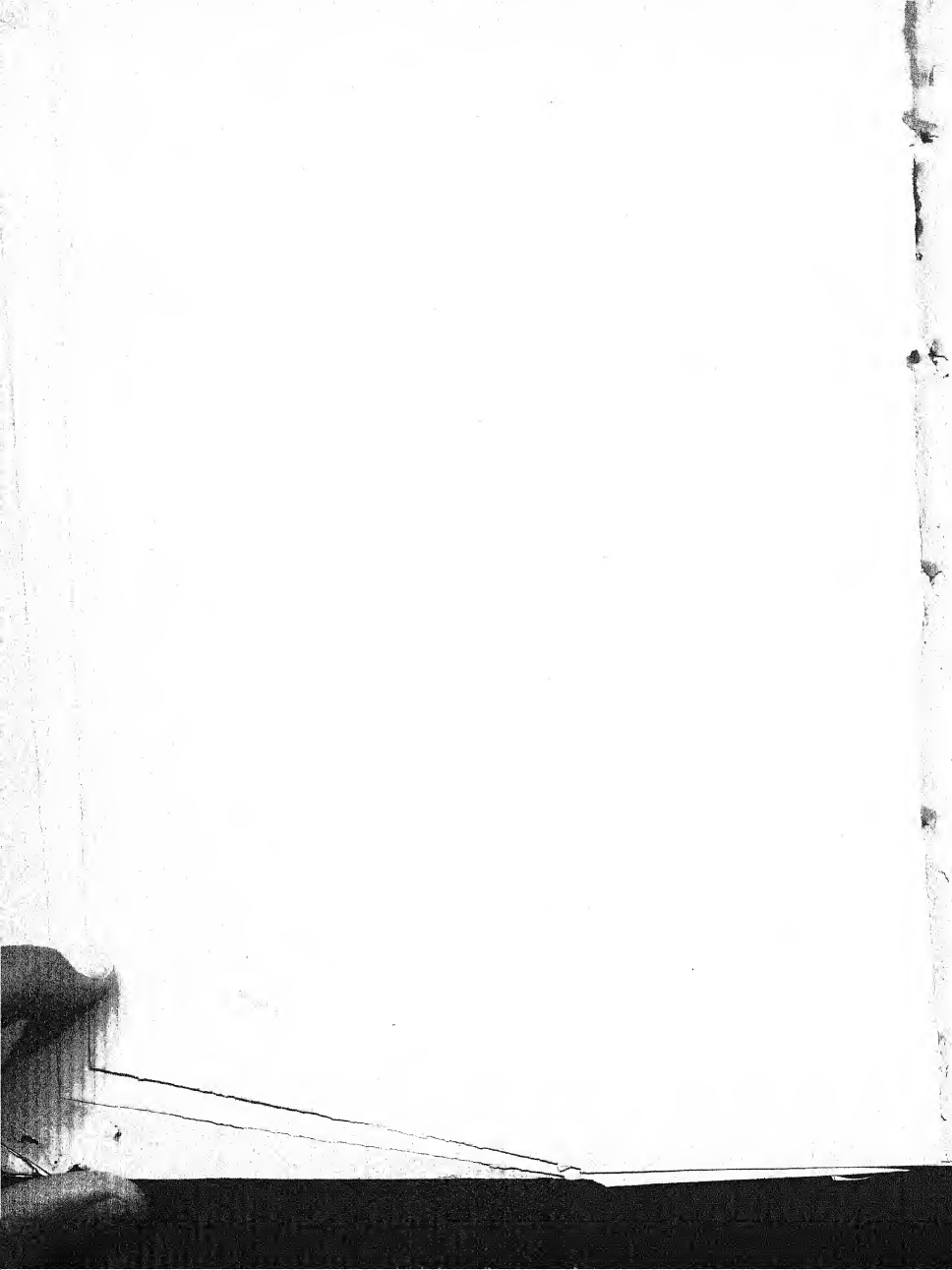
ALBERT HOWARD.

H. MARTIN LEAKE.

GABRIELLE L. C. HOWARD,

QUETTA,

August 5th, 1912.



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THE INFLUENCE OF THE ENVIRONMENT ON THE MILLING AND BAKING QUALITIES OF WHEAT IN INDIA.

No. 2. THE EXPERIMENTS OF 1909-10 AND 1910-11.

I. INTRODUCTION.

THE question of the influence of environment on the quality of wheat is of the greatest importance to those concerned with the improvement of this crop. It affects not only the question of seed distribution and the introduction of new varieties, but is also of vital interest to the breeder. It has long been a vexed question as to what extent quality in plant products is determined by environment and how far it must be considered as characteristic of the breed. To the plant-breeder, who wishes to combine in one race the largest number of valuable qualities, a knowledge of the respective parts played by breed and by environment in producing such qualities becomes essential. Further, in the general aspect of seed distribution in India it is essential to know in what tracts high milling and baking qualities are possible. For instance, at the present time, the black cotton soils of the Peninsula and the canal irrigated tracts of Northern India produce mainly soft weak wheats often with poor milling qualities. One of the objects of this investigation is to determine whether or not wheats of better quality can be grown in these important areas.

The experimental investigation of this subject in India was commenced in 1907 and the results obtained up to the harvest of 1909 have already been described.¹ Subsequently, the experiments

¹ Howard, Leake & Howard, *Memoirs of the Dept. of Agr. in India* (Botanical Series), Vol. III, No. 4, 1910, p. 191.

have been considerably extended, and in the present paper an account of the work done in the wheat growing seasons of 1909-10 and 1910-11 is to be found.

The characters of the wheat grain which may be affected by change of environment are the following:—

1. *Colour.* While the general colour, red or white, of any wheat remains the same no matter under what conditions it is grown nevertheless the depth or tone of colour in red or white wheats is not constant. In India, white wheats, when well grown under dry farming conditions, are frequently much darker in tint than the same wheat grown carelessly under a superabundant supply of canal irrigation. Similar differences are to be seen in red wheats.

2. *Size and weight of grain.* The size and absolute weight of the grain vary very considerably both in different localities in the same year and also in the same locality in different seasons.

3. *Composition.* Much of the work on the effect of environment on the characters of the wheat grain has been concerned with the effect of change of environment on the nitrogen content of the grain—the nitrogen content being taken not only as a measure of the percentage of gluten, but also as a rough indication of the strength of a wheat. There are, however, exceptions to the general rule that the higher the nitrogen, the greater the strength so that, in the present state of knowledge, the only safe method of estimating strength is by milling and baking tests. Quality as well as quantity of gluten is important in this respect. For a flour to be really strong there must be sufficient gluten of the right quality present. So far, while no accurate relation has hitherto been found between chemical composition and the bread-making value of wheat, nevertheless the trend of recent investigations on this subject affords hope that the strength of wheat may be explained from a chemical standpoint. Thus Wood has found in the case of Fife and other strong wheats that the water soluble phosphates in these flours is high—over 0.1 per cent. and the chlorides and sulphates very low. They also contain more magnesia than lime. Weak wheats on the other hand yield flours

with a low proportion of soluble phosphates, a high percentage of chlorides and sulphates with more lime than magnesia.¹

4. *Consistency.* The effect of environment on the consistency of the wheat grain, *i.e.*, its translucent or starchy appearance, has been perhaps more thoroughly investigated than any other aspect of the question. There is no doubt that consistency depends very largely on the soil, on the available moisture and on the nutrition of the crop and that, in many cases, great changes take place in this character in any variety according to the conditions under which it is grown. From the miller's point of view the consistency of the sample is of the highest importance and is one of the main factors in determining the value of wheat. Consistency is commercially important in two ways. Firstly, it affects the process of conditioning or the adjustment of water previous to grinding—as a rule translucent wheats take up more water than soft samples. Secondly, translucent grains usually behave better than soft wheats in the mill and are more free grinding, thus enabling the separation of bran from flour to be made with ease. As strong wheats are frequently translucent, translucency is sometimes considered to be an indication of strength, but this is not always the case as both translucent weak wheats and mellow strong wheats occur. In spite of these exceptions, however, the consistency of the grain remains a very important factor in the commercial valuation of a wheat.

5. *Quality.* Good quality in wheaten flour has been defined by Humphries² as “the sum of excellence on several points” and these are five in number: (1) flavour; (2) colour of the flour; (3) strength, *i.e.*, size and shape of loaf; (4) stability of dough; (5) yield of bread per sack of flour. It is obvious that information on such points cannot be determined in any other way than by milling and baking tests. In seeking information on the effect of environment on the quality of the grain it is therefore clearly essential to submit the sample to a complete test in the mill and bakehouse.

¹ See Jago—*The Technology of Breadmaking*, London, 1911, p. 323.

² Humphries, *Quality in Wheaten Flour*—a paper read before the Joint Session of the Chemistry, Botany and Agriculture Sections of the British Association at Winnipeg, 1909.

The earlier investigations carried out in Europe and North America, which bear on this subject, have been restricted to a great extent to the influence of external conditions on the composition of wheat. With the exception of the work of Humphries and Biffen¹ in England, little or no attention has been devoted to the more important practical question of the influence of environment on the milling and baking qualities. The earlier literature is referred to in the previous paper and, since this was published, several other contributions to the subject have appeared which are dealt with below.

In the United States, Le Clerc² has published a detailed account of his investigations on the influence of environment on the composition of wheat, the work being a continuation of previous experiments on this subject. Wheat from the same original seed was grown continuously at each of the three apices of two triangles (1) Kansas, Texas and California, (2) South Dakota, Kansas and California. The crop from each apex was then sent to the other two stations and then grown under the same conditions as the continuously grown seed. There were thus three plots at each station, all from the same original seed, one plot grown continuously at that point, the seed of the other two plots coming from the other apices of the triangle. Two samples of wheat were used in the experiments (a) Kubanka, a spring durum grown at South Dakota, Kansas and California and (b) Crimean, a common winter wheat, at Texas, Kansas and California. In the case of both wheats there were great variations in the percentage of nitrogen, in the consistency and in the absolute weight. The crop at each centre, no matter how different the composition and consistency of the seed sown, was practically identical. For example, when Kansas seed with 19.1 per cent. of protein, Texas seed with 12.2 per cent. of protein and the Californian seed with 10.4 per cent. of protein were all grown in California, the composition of the grain from the three plots was almost identical, namely, 11 per cent., 11.4 per cent. and 11.3 per cent. of protein.

¹ Humphries & Biffen, *Journal of Agricultural Science*, Vol. 11, 1907, p. 6.

² Le Clerc, *Bull.* 128, Bureau of Chemistry, U. S. Dept. of Agr., 1910.

The author concludes that the results indicate that soil and seed play a relatively small part in influencing the composition of crops and that the differences observed are due for the most part to climatic conditions prevailing at the time of growth. The results appear to be open to serious objection and are vitiated by the fact that the samples compared are by no means equally well grown. This is evident on examining the absolute weight of the same wheat grown at the various centres. The weight of 1,000 grains of Kubanka varied from 24.9 to 46.5 grammes while the limits of variation in the case of Crimean were 20.4 and 34.0 grammes. These variations are so great that they are bound to influence considerably the nitrogen content as, other things being equal, the lower the absolute weight the higher the nitrogen. Again, no milling and baking tests were made in these experiments and the conclusions are based entirely on the analytical data.

In Canada, Shutt has continued his investigations on the influence of environment on the composition of wheat, and the results are published in the *Report of the Canadian Experimental Farms* for 1909, 1910 and 1911. The experiments were conducted principally with Fife wheat at Lethbridge in South Alberta and are concerned with the influence of soil moisture on composition. In 1909 and 1910 especially, the samples from the non-irrigated land, in which the soil moisture decreased up to harvest time, contained a much higher percentage of nitrogen than those from the irrigated plots in which the soil moisture was appreciably greater. The samples do not seem to have been milled and baked so that it is not possible to determine how far the milling and baking characters have been influenced by the conditions under which the wheats were produced. Further, the absolute weight in the experiments of 1909 and 1910 is not stated, so that it is not possible to say whether the wheats compared were equally well grown or not.

Evans¹ has conducted some investigations bearing on the influence of soil moisture on consistency and composition in the Central

¹ Evans, *Bull. 3, Central Provinces Department of Agr.*, 1909.

Provinces of India. In durum wheats particularly it was found that the effect of excessive watering was to increase the percentage of soft and spotted grains and to lower the nitrogen content. The number of waterings given was nine and, in consequence, the crops often did not ripen till a month after the normal date. The treatment given was quite exceptional as in India it is not usual to water wheat nine times. Even in the Canal Colonies of the Punjab, where the land was previously a desert, three waterings only is the rule. Speaking generally, irrigation for wheat on the black soil of the Central Provinces is not common as the soil usually contains sufficient moisture for the wheat crop. In these experiments the absolute weight of the sample is not stated so that it is impossible to interpret the figures for the nitrogen content with accuracy.

Leather¹ has published a preliminary note on the effect of manures on the composition of wheat and other grains but no details of the experiments are given.

As in the older work on this subject the recent investigations referred to are all characterised by the absence of milling and baking tests. In order to obtain accurate information on the effect of environment on the behaviour of the same sample in the mill and subsequently in the bakehouse it is, in the present state of knowledge, unsafe to rely on chemical data only and on the appearance of the samples. Such important matters as strength of flour and the freemilling nature of the wheats might easily be masked by changes in consistency and nitrogen content. That this is so is proved by the results described in the present paper.

The agricultural conditions under which wheat is grown in India have been referred to in detail in the previous paper on this subject. India possesses two great wheat tracts which differ from each other both as regards soil and as regards the source of moisture. The more important of these regions is the alluvium of the Indo-Gangetic plain, stretching from Bihar on the East through the United Provinces and the Punjab to Sind on the Western coast.

¹ Leather, *Seventh International Congress of Applied Chemistry*, Section VII, 1909, p. 157.

In parts of Bihar, wheat is grown on high moisture retaining heavy loams without irrigation. In Oudh, wells supplement the rainfall, while in the Doab, between the Jumna and the Ganges, and also in the Western Districts of the United Provinces canal water is commonly employed. In the Punjab, the wheat crop is largely watered from perennial and inundation canals while, in Sind, inundation from the Indus takes the place of the monsoon. The predominant features of the wheat tracts of the plains are the alluvial character of the soil and the occurrence of some form of irrigation. The second great wheat growing tract in India is found in the Peninsula on the black cotton soils of the Central Provinces and Bombay. Here irrigation is the exception and most of the wheat is grown on the moisture left in the soil after the previous monsoon.

Besides moisture and soil another factor in Indian wheat production is of importance. This is the length of the growth period. Wheat is generally sown as soon as the temperature falls sufficiently for germination to take place and for the seedlings to develop. The length of the growth period in the spring is also limited by temperature and the rapid advance of the hot season prevents the cultivation of late maturing wheats. The growth period is shortest in Central India and longest in the Punjab and North-West Frontier Province.

The main directions in which Indian wheat can be improved are two—yield and quality. The shortness of the growth period and the fact that the water supply is liable to be deficient in amount indicate that moderate yielding wheats are likely to be the most profitable to the grower over an average of seasons. Higher yielding wheats can be grown with safety only where the supply of moisture is adequate and where the retentive power of the soil is considerable. Compared with the high yielding wheats of Western Europe, where the growth period is long and the rainfall well distributed, the wheats of India are but moderate yielders. Generally speaking, the season is too short in India for the growth of such high cropping wheats as those of France and England. As yield is determined by the length of the growth period and the average water supply, the plant

breeder in increasing the amount of wheat grown, soon reaches the limit imposed by these conditions. In the improvement of the quality of the grain of Indian wheat however there is much greater scope for the breeder. In general, the wheats of the country have poor grain qualities, both from the milling aspect and also from the point of view of breadmaking. Some Indian wheats do not mill well while the majority have a reputation for producing weak flour.

In the improvement of the quality of Indian wheat it is of the first importance to determine how far grain qualities are influenced by environment, and in what tracts high quality wheats retain their characters. Accurate information on the character of the wheat produced in the various tracts and the extent of these tracts will enable a scientific scheme of wheat distribution to be drawn up for the whole of India. It is sometimes stated that high quality wheats cannot be grown either on the black cotton soils or under canal irrigation in the Indo-Gangetic plain, particularly in the United Provinces and the Punjab which produce the bulk of the wheat exported. One of the main objects of these experiments is to test these views and to determine to what extent, if any, canal irrigation interferes with high milling and baking qualities.

The method adopted in this investigation is to compare, as regards consistency, absolute weight, nitrogen content and milling and baking qualities, several pure lines, of widely different quality, grown at various stations. The stations have been selected so as to include as many as possible of the most important wheat tracts of the Indo-Gangetic plain as well as a few places representative of the black cotton soil areas of Peninsular India. When a sufficient number of well-chosen types of wheat, representative of the various classes grown, have been tested in this manner it will be possible to determine how far milling and baking qualities are affected by environment and whether such a change is progressive or whether it takes place once for all.

In the previous paper the results obtained in 1908 and 1909 were described. In both years it was found in the case of Muzaffar-

nagar (a soft, white, weak wheat), grown at different stations in the alluvium, that the samples differed widely in consistency, nitrogen content and to some extent in the milling and baking tests. The results of the two years are summed up in the following tables.

Consistency, composition and baking value of Muzaffarnagar white grown at three stations in 1907-1908.

Where grown.	Colour and Consistency.	% Nitrogen. (Leather).	Order in baking value (Humphries).
1. Pusa	Amber, mostly hard and semi-hard ..	1.86	Fifth.
2. Lyallpur ..	Dull white, mostly soft and mottled	1.50	Eighth.
3. Muzaffarnagar ..	White, entirely soft	1.38	Ninth.

Comparative value of Muzaffarnagar white grown at nine stations in 1908-1909.

Where grown.	CONSISTENCY.			Weight of 1,000 grains in grammes.	Nitrogen percentage.	BAKERS' MARKS.		Order of merit in commercial value.
	Soft.	Intermediate.	Hard.			Stability.	Strength.	
Cawnpore ..	8	48	39	35.00	2.37	84	75	First.
Pusa ..	16	59	25	38.35	2.00	84	75	Second.
Partabgarh ..	36	33	31	40.02	1.79	82	75	Third.
Dumraon ..	72	21	7	43.07	1.52	78	63	Fourth.
Algarh ..	76	15	9	42.54	1.30	70	60	Fifth.
Meerut	4	38.95	1.34	70	60	Sixth.
Bankipore ..	83	13	..	37.08	1.34	68	52	Seventh.
Lyallpur ..	75	20	5	37.08	1.52	68	68	Eighth.
Orai ..	95	0	0	30.97	1.93	64	66	Ninth.

In addition to the tests with Muzaffarnagar white, twenty-five Punjab wheats, belonging to three subspecies, were grown in pure line culture at Cawnpore and compared with the same wheats grown the previous year at Lyallpur. In general, there was a large increase in the proportion of hard grains at Cawnpore and also an increase in the percentage of nitrogen. These wheats have since been grown for two seasons at Cawnpore and at Pusa, and the complete results are described in the next section.

II. THE EFFECT OF ENVIRONMENT ON THE TYPES OF PUNJAB WHEAT.

In 1908, the types of wheat grown in the Punjab were botanically and agriculturally classified at Lyallpur and twenty-five distinct wheats were distinguished. This work has been described in a previous paper.¹ Subsequently, these wheats were grown for three years at Cawnpore and for two years at Pusa. The consistency of the samples, the nitrogen percentage, and the weight of 1,000 grains have been determined and the figures, compared with those obtained from the original seed grown at Lyallpur in 1907-08, are given in the table below.

In considering the results of the determinations of the consistency of the samples it should be remembered that, apart from the influences of the soil and climate, the agricultural conditions under which the samples have been grown at the three stations are not strictly comparable. At Lyallpur, the wheat crop is overwatered and the methods of cultivation are primitive. At Cawnpore, great care is taken to prevent overwatering while at the same time the methods of cultivation have attained a high degree of efficiency. At Pusa, wheat is grown as a dry crop and particular attention has been paid for many years to preliminary cultivation, to the conservation of soil moisture and to the eradication of weeds. The general appearance of the same wheat grown at Cawnpore and Pusa is very similar and the tendency at both stations is to grow similar uniform translucent bright wheats, which take the eye and contain a high percentage of nitrogen. At Lyallpur on the other hand, the production of dull soft samples with a low nitrogen content is the rule. These general results are clearly indicated in the table and

¹ Howard & Howard, *Memoirs of the Department of Agriculture in India (Botanical Series)*, Vol. II, No. 7, 1909.

in the majority of the types there is a distinct increase in the proportion of translucent grains at Cawnpore and Pusa while at the same time the percentage of nitrogen has increased.

There are three well marked exceptions to the hardening tendency among these wheats. Type 6 has always remained entirely soft at all three stations and no spotted grains have ever been detected. Types 23 & 25 have behaved in a very similar manner and have only rarely produced a few hard and intermediate grains. These examples stand out from the many varieties grown at Pusa in withstanding the hardening tendency which is the general rule at this station. Even in 1911, when the water supply was very short, these wheats, though shrivelled, contained no hard grains. On the other hand, Types 2 & 12 and to a less extent Type 3 have produced a great majority of hard grains at all three stations. Between these two extremes the remaining nineteen types occur producing a considerable proportion of soft grains at Lyallpur and a lesser proportion at Cawnpore and Pusa. Thus there are three classes of wheats among these types, (1) wheats which always remain soft, (2) wheats with a tendency to remain hard and (3) the majority in which the consistency varies greatly according to the locality and the conditions under which they are grown. In the wheats of the last group, as has frequently been observed before, each ear often contains hard, soft, and spotted grains so that the occurrence of all three kinds of grain in the sample is not likely to be due to the result of segregation.

The occurrence of constant soft wheats is of considerable interest and has been noticed in other pure lines at Pusa. One of these, characterised by strong straw, has been used as a parent for improving the standing power of one of the high quality hard wheats. In the second generation of the cross there was a distinct segregation as regards consistency and plants with hard, soft and intermediate grain could be distinguished with ease and certainty. Two other cases of wheats with constant soft grains are of interest from the milling point of view. One of these is that known as "Australian 27" which is largely composed of Punjab Type 25, a type which has

remained soft at Cawnpore and Pusa. The second is a Pusa pure line (No. 3) which has always produced a soft sample. Both these wheats behaved badly in milling and were described as woolly in texture and gave great trouble in the separation of flour and bran. As it is possible that some connection may exist between these bad milling qualities and the tendency to produce a soft sample under all conditions steps are being taken to have complete milling tests made of Punjab Types 6, 23 & 25 when grown at Cawnpore under conditions where most Indian wheats produce samples which mill exceedingly well. At the same time the study of the inheritance of the constant soft character is being continued at Pusa.

The nitrogen content of these constant soft wheats is not without interest. In no case is the percentage really low while in others it is remarkably high as will be seen in the table below. There appears to be no connection between nitrogen content and the tendency to produce soft grains.

Nitrogen content of constant soft wheats.

<i>Name.</i>	<i>Average weight 1,000 grains.</i>	<i>Average nitrogen percentage.</i>
Pusa 3	48.95	2.42
A 88	...	2.21
Punjab Type 6	29.75	1.83
" " 23	32.63	1.80
" " 25	40.97	1.70

In the case of the three Punjab Types the figures are the average of five determinations.

The change in consistency of the 25 types of Punjab wheat when grown at Cawnpore and Pusa.

BOTANICAL VARIETY.		CONSISTENCY.															NITROGEN PERCENTAGE.						WEIGHT OF 1,000 GRAINS IN GRAMMES.																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																							
		LYALLPUR 1907-08.			CAWNPORE 1908-09.			CAWNPORE 1909-10.			CAWNPORE 1910-11.			PUSA 1909-10.			PUSA 1910-11.			LYALL- PUR.	CAWNPORE.			PUSA.		LYALL- PUR.	CAWNPORE.			PUSA.																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																
		Soft.	Intermediate.	Hard.	Soft.	Intermediate.	Hard.	Soft.	Intermediate.	Hard.	Soft.	Intermediate.	Hard.	Soft.	Intermediate.	Hard.	Soft.	Intermediate.	Hard.		1907-08.	1908-09.	1909-10.	1910-11.	1907-08.		1908-09.	1909-10.	1910-11.	1907-08.	1908-09.	1909-10.	1910-11.																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
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* Consistency uniform but intermediate between hard and soft.





III. THE INFLUENCE OF ENVIRONMENT ON GRAIN QUALITY.

A. THE RESULTS OBTAINED IN 1909-10.

IN this section the results obtained by growing the same wheat at different stations and comparing the produce are dealt with. During the season 1909-10, two wheats were grown at the ten stations referred to below and complete milling and baking tests were made in all cases. The two wheats selected were Muzaffarnagar white, (*T. vulgare* Vill. var. *graecum* Kcke.) a weak, soft, white wheat from the Muzaffarnagar District in the United Provinces and Pusa 20, a pure line selected from a Bengal durum wheat, (*T. durum* Desf. var. *apulicum* Kcke.) The experiments with Muzaffarnagar white are in continuation of those described in the previous paper, and the seed sown at each station was taken from the crop of the previous year grown at that station. In the case of Pusa 20 the seed was taken from the bulk grown at Pusa. The ten stations at which these wheats were grown were :—

1. *Pusa*. This station is situated in North Bihar, a tract in which wheat is grown without irrigation on high moisture retaining loams containing about 30% of calcium carbonate.
2. *Cawnpore*. At this station, which is typical of the important wheat tract known as the Middle Doab, wheat is grown on strong loams with canal irrigation.
3. *Partabgarh*. This station represents the wheat growing tracts of Oudh where the crop is grown on well irrigation.
4. *Meerut*. This station, where the samples were grown by a local *zemindar* in the country fashion, is typical of the large wheat growing tract of the Upper Doab and is practically identical with the neighbouring Muzaffarnagar District.

5. *Aligarh*. The conditions at this station are similar to those obtaining in the Meerut District. This centre is therefore a duplicate of the above and both are situated in canal irrigated tracts.

6. *Hoshangabad*. This centre, which is situated outside the Indo-Gangetic alluvium, is characteristic of the black cotton soil of Peninsular India on which wheat is grown year after year on the moisture stored up in the soil from the previous monsoon. Both Muzaffarnagar white and Pusa 20 did not grow to perfection on the soil moisture available and proved to be late varieties for this tract.

7. *Orai*. This station, which is also outside the Gangetic alluvium, is situated in Bundelkhand where wheat is grown on moisture retaining clays which are however sometimes watered.

8. *Bankipore*. This station is situated in Bihar on the south bank of the Ganges, but the soil is not alluvial in character. It is a heavy moisture retaining clay not unlike some of the soils of Peninsular India.

9. *Dumraon*. This station occurs in the wheat growing tracts in South Bihar. The soil is sandy and possesses little or no moisture retaining capacity. In consequence even with irrigation the yields of grain and straw are small.

10. *Lyallpur*. This station is typical of the canal irrigated tract known as the Chenab Colony which produces a large proportion of the wheats exported from Karachi. Overwatering is common while the general standard of cultivation for the wheat crop is below that practised by the best cultivators in the Eastern Punjab and in the Upper Doab.

Trial of Muzaffarnagar White at ten stations, 1909-10.

	PUSA.	CAWNPORE.	PARTABGARH.	MERRUT.	ALIGARH.	HOSHANGABAD.	OPAI.	BANKIPORE.	DUMRAON.	LYALLPUR.
1. Type of soil	Second class loam.	Loam (<i>dumai</i>).	Loam (<i>dorasa</i>).	Loam.	Light loam.	Black cotton soil.	Typical wheat soil (<i>mar</i>).	Heavy clay.	Sandy loam.	Sandy loam.
2. Previous crop	Gram in <i>rabi</i> of 1908-09 ploughed in.	Mixed crop of <i>arhar</i> and <i>juar</i> .	Sugarcane.	Sugarcane.	Cotton.	Wheat.	Gram.	<i>Juar</i> (crop failed).	<i>Aus</i> paddy.	Wheat.
3. Manuring	Green manured with gram in the previous <i>rabi</i> .	Nil.	Nil.	Nil.	Poudrette containing 50 lbs. nitrogen per acre.	Nil.	Nil.	Nil.	Cowdung at the rate of 83 mds. per acre on 17th October 1909.	Nil.
4. Preparation for wheat	Exposed several times by iron ploughs during previous hot season, afterwards fallowed.	Ploughed six times before sowing with iron ploughs on 1st April, 15th June, 5th Aug., 14th, 20th & 29th Sep. 1909.	Ploughed four times with iron ploughs on 2nd, 18th Aug., 12th Sep. and 2nd Oct. 1909, and three times with the country plough on 15th Aug., 8th and 15th Oct. 1909.	Ten ploughings with country plough after beginning of the rainy season.	Three ploughings with iron ploughs (28th Jan., 14th July, 9th Aug. 1909), and five times with the country plough (17th and 28th Aug., 13th and 18th Sep. and 8th Oct. 1909).	Ploughed once with country plough and scarified four times with the <i>bakkar</i> .	Ploughed once with country plough (17th May 1909), twice with iron plough (21st Aug., 13th Sep. 1909) and scarified once with <i>bakkar</i> (30th Sep. 1909).	Ploughed eight times between 27th and 29th Oct. 1909.	Ploughed seven times before sowing between 12th Sep. and 28th Oct. 1909.	Ploughed four times with the native plough. One irrigation during preliminary preparation.
5. Date and method of sowing	18th Oct. 1909, behind the country plough.	24th Oct. 1909, behind the country plough.	17th Oct. 1909, behind the country plough.	9th Nov. 1909, behind the country plough.	31st Oct. 1909, behind the country plough.	18th Oct. 1909, with the country drill.	15th Oct. 1909, behind the country plough.	29th Oct. 1909, with the local drill.	27th Oct. 1909, with the local drill.	6th Nov. 1909, after the country plough.
6. Seed rate (lbs. per acre)	55	120	108	90	100	95	108	100	100	48
7. Rain. (April 1st to fall in inches)	75.37	30.8	50.21	33.52	21.34	32.4	44.74	46.93	46.25	20.41
During growth period	0.22	1.81	1.40	2.59	2.28	1.07	0.58	0.00	1.04	2.00
8. Irrigation	Once, December 1909.	Twice from canal on 28th Nov. 1909 & 28th Jan. 1910.	Twice from well on 17th Nov. & 10th Dec. 1909.	Twice.	Three times (17th Nov. 1909, 2nd Feb. & 8th March 1910).	Nil.	Twice (7th Dec. 1909 & 9th Feb. 1910).	Once (21st Nov. 1909).	Three times (3rd Dec. 1909, 8th Feb. & 8th March 1910).	Four times—once by accident in April, ten days before harvest.
9. Date of harvesting	26th March 1910.	6th April 1910.	29th March 1910.	14th April 1910.	16th April 1910.	16th March 1910.	1st April 1910.	30th March 1910.	4th April 1910.	21st April 1910.
10. Growth period in days	162	164	163	156	167	149	168	145	159	166
11. Consistency of sample	0% Soft 57% Intermediate 43% Hard	5 72 23	50 38 12	54 30 16	57 35 8	66 7 27	66 29 5	79 14 7	58 36 6	73 16 6
12. Nitrogen percentage	2.19	1.79	1.49	1.79	1.60	2.05	1.45	1.48	1.41	1.38
13. Weight of 1,000 grains in grammes	39.80	38.07	39.71	37.92	34.58	33.30	37.65	42.58	40.73	40.47
14. Order of merit in commercial value	First.	Second.	Third.	Fourth.	Fifth.	Sixth.	Seventh.	Eighth.	Ninth.	Tenth.

1. Type of soil ..

2. Previous crop ..

3. Manuring ..

4. Preparation for
wheat

5. Date and method of
sowing

6. Seed rate (lbs. per
acre)

7. Rain- (April 1st to
fall in { sowing time,
inches { During growth
period

8. Irrigation ..

9. Date of harvesting

10. Growth period in
days

11. Consist- { % Soft
ency of { % Inter-
sample { mediate
{ % Hard

12. Nitrogen percent-
age

13. Weight of 1,000
in grammes

14. Merit in
commercial value

39.8

First.

the plains of India wheat comes to perfection only if the soil has been well prepared beforehand and when this preparation extends over a sufficiently long period. To obtain optimum results, both as regards yield and quality, this preparation should extend over the previous hot season as well as during the rains. It is only when the soil has been exposed to the sweetening influences of the sun and air during the hottest part of the year in April and May (which process also kills out most weeds) and subsequently cultivated during the monsoon that the maximum amount of moisture can be absorbed and the soil brought into the highest condition of fertility for wheat. A hurried preparation always adversely affects the yield and is also reflected in the poor general appearance, low nitrogen content and in the milling and baking results. On the other hand, where great attention is paid to cultivation, clean weeding and moisture conservation, the samples are bright and well grown and take the eye. They also tend to contain a high percentage of nitrogen and invariably do well in the milling and baking tests.

Muzaffarnagar White.

For the season 1909-10 the details relating to the trial of this wheat at the above ten stations are to be found in the table opposite and the result of the milling and baking tests are referred to in the following extract from Mr. A. E. Humphries' report, dated October 27th, 1910.

Muzaffarnagar.

"I understand all ten lots were produced from the same seed but that each one was grown at different places, Pusa, Bankipore, Dumraon, Partabgarh, Cawnpore, Orai, Aligarh, Meerut, Lyallpur and Hoshangabad. In 1909, I tested sample lots of this wheat grown at the nine places mentioned first in the list. I note that the Hoshangabad sample represents a lot grown experimentally in a new environment. There were great differences between the 1909 lots which would represent "several shillings per quarter." The lot in that season from Orai was a "dingy looking shrivelled wheat,"

the Lyallpur lot had a poor appearance but yielded better flour and bread than its appearance would lead one to expect. This year the ten lots do not show such great differences in appearance and all of them may be described as good looking specimens of the variety. The Hoshangabad lot contains a considerable percentage of light grains and some greenish or actually green ones. It will be interesting to know whether these blemishes were caused by early cutting or by the wheat maturing too late for the locality. Be that as it may, it is nevertheless a good lot of wheat. The Bankipore lot also contains a few greenish corns and the same ideas suggest themselves but in lesser degree. The Hoshangabad, Aligarh and Dumraon lots are smaller in the berry than the other lots, but a miller who wanted soft, weak wheat, suitable for making biscuit or pudding flours, or who wanted some soft weak flour for toning down the harshness of other kinds, would be very hard to please if he were not content with any one of these lots. The Pusa lot is particularly well grown. In buying wheats, especially those which are not in large regular supply a miller has to base his ideas as to commercial value on many points by appearance and before I had milled and baked any of these lots I had placed them in the following order, and assessed the difference in money value between the best and worst at 1/-per quarter only. It may be interesting to place last year's order in juxtaposition.

1909.			1910.
Cawnpore	Pusa.
Pusa	Cawnpore.
Partabgarh	Partabgarh.
Dumraon	Meerut.
Aligarh	Aligarh.
Meerut	Hoshangabad.
Bankipore	Orai.
Lyallpur	Dumraon.
Orai	Lyallpur.

In view of remarks which I make at the end of the report concerning the influence of water supply on quality it may be worth

mentioning that the moisture content of the Lyallpur lot was very low on its arrival here, but I attach little importance to that under the circumstances, for the others may perhaps have taken up water from the atmosphere in transit. The lots from Pusa, Dumraon and Lyallpur are harder than the remainder; but none of them are hard, all are in the category of soft wheats, and in milling them the moisture content would have to be kept relatively low if satisfactory separations are to be made easily. The Orai, Aligarh and Meerut lots are distinctly "woolly" in texture, whereas the Dumraon lot, in spite of its pale "starchy" appearance, behaves notably well for the variety in milling. The outstanding features of the bakehouse results are three.

1st. The superiority of the Pusa lot, which tallies with its appearance as specified hereinbefore.

2nd. The way in which the Pusa and Hoshangabad responded when a form of malt extract was added.

3rd. The dull uniformity of the remainder in yielding poor miserable loaves under all conditions.

No one used to eating average London bread would buy such poor stuff as the eight poorest yielded. The exterior appearance was wretched, in the Lyallpur case particularly so, for in addition to the chalky-hued, hard, thick crust, characteristic of the variety, that lot exhibited the peculiar cracking of the crust characteristic of English Rivet wheat. The loaves from the eight did not expand in the oven, so they were of very small volume. The flavour of the bread was poor or definitely bad. The case of the Hoshangabad lot was remarkable. At the first baking when the dough was made with flour, yeast, salt and water only, it was certainly better than the eight lots already referred to, but it was not good. When, however, a form of malt extract was added in the second baking a transformation was effected. The loaf was larger, and of pleasing appearance, its flavour was very greatly improved. It is obvious that this lot possessed potentialities which would be readily developed by skilful treatment at the hands of the miller or baker, whereas so far as I have been able to go, on the quantities of wheat

available this season, the eight lots do not seem to possess those potentialities. The Pusa lot was much superior to the remainder throughout the baking trials. It responded to the addition of malt extract but not to so great an extent as the Hoshangabad lot. This means, that at the first baking the former was much superior to the latter and that at the second baking the former was still superior to the latter, but not in the same degree.

I have already in this as well as in former reports said that this variety of flour is quite suitable for pudding or cake making; indeed, for such purposes it would probably be superior to other varieties much more suitable for bread making. For pudding and similar purposes, it would be judged by its appearance as flour, and its poor or bad flavour would be disguised by the fats, mineral salts and spices mixed with it. For bread making purposes the Pusa stands out as being very much the best lot, but the flour itself produced from it is not so white as that produced from seven of the other lots. Of Hoshangabad the same remark can be made. That flour has a yellow hue. The Lyallpur lot has a dingy hue. The last named lot I would therefore now unhesitatingly place last in the list. It is poor both as flour and as bread, and that in my opinion overrides the superiority it shows in the milling processes."

In a subsequent communication, dated December 8th, 1909, Mr. Humphries has forwarded the bakers' marks of the ten samples of Muzaffarnagar. The results are as follows:—

	<i>Without malt extract.</i>		<i>With malt extract.</i>	
	Stability.	Strength.	Stability.	Strength.
Pusa	80	72	88	79
Hoshangabad	74	62	82	81
Cawnpore	72	62	72	64
Meerut	70	62	70	64
Aligarh	70	62	70	62
Dumraon	72	62	75	60
Partabgarh	70	62	66	60
Orai	72	62	70	60
Bankipore	72	62	70	60
Lyallpur	70	62	68	60

Trial of Pusa 20 at ten stations, 1909-10.

	PUSA.	HOSHANGABAD.	ALIGARH.	CAWNPORE.	DUMRAON.	MEERUT.	ORAI.	BANKIPORE.	PARTABGARH.	LYALLPUR.
1. Type of soil	Second class loam.	Black cotton soil.	Light loam.	Loam (<i>dumat</i>).	Sandy loam.	Loam.	Typical wheat soil (<i>mar</i>). Gram.	Heavy clay.	Loam (<i>dorasu</i>).	Sandy loam.
2. Previous crop	Gram in <i>rabi</i> of 1908-09 ploughed in.	Wheat.	Cotton.	Mixed crop of <i>arhar</i> and <i>jaar</i> .	<i>Aus</i> paddy.	Sugarcane.		<i>Jwar</i> (crop failed).	Sugarcane.	Wheat.
3. Manuring	Green manured with gram in the previous <i>rabi</i> .	Nil.	Poudrette containing 50 lbs. nitrogen per acre.	Nil.	83 mds. cowdung per acre on 17th October 1909.	Nil.	Nil.	Nil.	Nil.	Nil.
4. Preparation of wheat	Exposed several times by iron ploughs during previous hot season, afterwards fallowed.	Ploughed once with country plough, scarified four times with the <i>bakhar</i> .	Three ploughings with iron plough (28th Jan., 14th July, 9th Aug. 1909), and five times with country plough (17th and 28th Aug., 13th and 18th Sept., and 8th Oct. 1909).	Six ploughings with iron plough on 1st April, 15th June, 3th Aug., 14th, 20th and 29th Sep. 1909.	Ploughed seven times before sowing between 12th Sep. & 26th October 1909.	Ten ploughings with country plough after the beginning of the rainy season.	Ploughed once with country plough (17th May 1909), twice with iron plough (21st Aug., & 13th Sep. 1909), and scarified once with the <i>ba bhar</i> (30th Sep. 1909).	Ploughed eight times between 27th and 29th Oct. 1909.	Ploughed four times with iron plough (2nd and 18th Aug., 2nd Oct. 1909), three times with the country plough (15th Aug., 8th and 15th Oct. 1909).	Ploughed four times with the native plough. One irrigation during preliminary preparation.
5. Date and method of sowing	18th Oct. 1909, behind the country plough.	18th Oct. 1909, with the country drill.	31st Oct. 1909, behind the country plough.	24th Oct. 1909, behind the country plough.	27th Oct. 1909, with the local drill.	9th Nov. 1909, behind the country plough.	15th Oct. 1909, behind the country plough.	26th Oct. 1909, with the local drill.	17th Oct. 1909, behind the country plough.	6th Nov. 1909, behind the country plough.
6. Seed rate (lbs. per acre)	55	95	100	120	100	60	108	100	108	48
7. Rain, { April 1st to fall in { { sowing time { { During growth { { period { { inches {	75.37 0.22	32.40 1.07	21.34 2.28	30.82 1.81	40.25 1.04	33.52 2.50	44.74 0.58	46.98 0.00	50.21 1.40	20.41 2.09
8. Irrigation	Nil.	Nil.	Four times (17th Nov. 1909, 2nd Feb., 6th & 28th March 1910).	Twice from canal (28th Nov. 1909, 28th Jan. 1910).	Three times (3rd Dec. 1909, 8th Feb., & 8th March 1910).	Three times.	Twice (7th Dec. 1909 & 9th Feb. 1910).	Once (21st Nov. 1909).	Twice from well (17th Nov. 1909 & 10th Dec. 1909).	Four times. Once by accident 10 days before harvest.
9. Date of harvesting	28th March 1910.	10th March 1910.	16th April 1910.	3rd April 1910.	4th April 1910.	14th April 1910.	1st April 1910.	30th March 1910.	29th March 1910.	21st April 1910.
10. Growth period in days	161	149	167	161	159	156	168	145	163	166
11. Con- { sistency { { of { { sample { { % Soft { { % Inter- { { mediate { { % Hard {	0 0 100	0 10 90	0 4 96	0 1 99	0 16 84	6 25 69	0 13 87	0 13 87	0 18 82	11 55 34
12. Nitrogen percentage	2.64	2.47	2.01	2.26	1.70	2.06	1.90	1.81	1.69	1.72
13. Weight of 1,000 grains in grammes	42.37	33.87	31.86	32.66	41.12	30.04	35.56	41.91	38.06	30.35
14. Order of merit in commercial value	First.	Second.	Third.	Fourth.	Fifth.	Sixth.	Seventh.	Eighth.	Ninth.	Tenth.



Pusa 20.

The details relating to the trial of this wheat at the ten stations are given in the table opposite and the results of the milling and baking tests are described in the following extract from Mr. A. E. Humphries' report of 1910.

"If it be desirable in the interests of the producer to grow Durum wheats in India, I think it would be desirable to encourage the distribution of this variety of that type. The latest developments in the art of milling minimize to some extent the objections of millers to Durums, but those objections persist and are likely to do so. But there are Durums and Durums, and this variety is a good one. At its best it differs very little in appearance from some of the best hard 'ordinary' wheats, and in milling it, I have found with very much interest that we have obtained, without any special effort, on an average of the ten samples in each case a rather higher percentage of flour than we obtained from the Muzaffarnagars. This to a miller is very noteworthy.

The ten samples were grown at the same places as the ten samples of Muzaffarnagar. In the former set there are the same sort of differences as in the latter. Here also the Hoshangabad lot contains a proportion of thin grains and some which are green. The Cawnpore also contains a few thin grains, but on this point the Meerut lot contains a much larger proportion and a substantial proportion of pale corns besides. The Lyallpur contains a still larger proportion of pale corns and is the poorest of these lots in general appearance. In these ten samples also we get differences in hue, some of a much deeper yellow than others. In the Pusa Muzaffarnagar the deeper yellow hue improves its appearance, but in the Pusa Durum the intensity of its yellow hue detracts from its looks. The Pusa Durum is also very regular in its texture and is particularly well grown.

In spite of these blemishes all the lots with the exception of Meerut may be described as well grown. The Pusa lot appeared on first examination to be hardest, but after conditioning the Cawnpore was found to be so. Judged by appearance only, I should

consider the best worth one and sixpence per quarter more than the worst, and I should place them in the following order:—

Dumraon }
Orai }
Bankipore }
Cawnpore }
Partabgarh
Aligarh
Pusa
Hoshangabad
Meerut
Lyallpur.

The subsequent baking trials in these cases caused me to change my opinions, but I am putting my first impressions on record for they would be the basis upon which I should make, at least the earliest, purchases of such wheat. Their behaviour in milling calls for no special comment beyond the one already made. Such flours as these are not at all likely to be sold by appearance, but it may as well be recorded that they are, even after conditioning of the wheat, very granular, that the Meerut and Aligarh lots have a very yellow hue, that the Dumraon and Orai lots are the best judged by appearance and Pusa the worst. When baked into bread Pusa again shows a great superiority with Hoshangabad a good second. From the Durums of commerce, one does not usually obtain tough doughs nor do eight of these lots behave in that way, but the Pusa lot does so and so far as toughness of dough is concerned the Hoshangabad lot is as good. The Pusa yields however decidedly the best loaf, the Hoshangabad is not far behind, the Lyallpur loaf is decidedly the worst with low volume and a poor crust; the Meerut is almost as bad. The other six lots are, within the margin of experimental error, alike. The flavour of the bread produced from each one of them is very pleasant. In these ten cases I made no attempt to add yeast foods or similar materials. It seemed to me desirable to get this set of baking trials made in the simplest way."

In a subsequent communication, dated December 8th, 1910, Mr. Humphries forwarded the bakers' marks of the above wheats. The results were as follows :—

		<i>Stability. Strength.</i>		
Pusa	.. 88	80	Makes tough doughs of a style of toughness which we expect to get in American spring wheat.	
Hoshangabad..	80	74	In lesser degree has the same toughness in dough as the Pusa lot.	
Aligarh	.. 75	65	Cannot be described as tough in dough.	
Cawnpore	.. 75	65	Ditto.	
Meerut	.. 70	64	Lacks toughness in dough.	
Dumraon	.. 70	64		
Orai	.. 65	62	Yields a poor crust similar in character to our English Rivet wheat.	
Bankipore	.. 70	62		
Partabgarh	.. 65	60	Dough has very little tenacity.	
Lyallpur	.. 70	57	Yields a very fairly tough dough, but a miserable loaf, with a cracked "rivety" crust of very poor appearance.	

In the following season, 1910-11, samples of this wheat from the ten stations were grown side by side at Pusa and then compared in all respects. No differences could be distinguished in the plots themselves which behaved exactly alike in the field and the crop to the eye was absolutely level. As regards the grain the samples were alike in appearance and consistency and, as was expected, none of the differences noted in the original samples sown manifested themselves at harvest time. In this case the environmental differences disappeared as soon as the wheat was brought back to its original home, thus confirming Le Clerc's observations on this point in the United States.

B. THE INFLUENCE OF ENVIRONMENT ON STRENGTH.

It will be seen in the extensive trials of the weak wheat from the Muzaffarnagar District that in no case did this variety become strong. All that occurred was that at stations like Cawnpore and Pusa, where particular attention has been paid to growing the wheat crop to perfection, the sample improved both in appearance and also in the milling and baking processes. The wheat, however, still behaved as a weak wheat and exhibited all the characteristics of its class.

The behaviour of wheats, characterised by strength of flour and good milling qualities, when grown under similar conditions to Muzaffarnagar, must now be considered. For the purpose of ascertaining how far strength and other desirable grain qualities are maintained, four types of strong wheat (Pusa 4, 8, 12 & 22) were selected. In addition, a sample of Muzaffarnagar was also grown at each centre, so that the strong types could be compared with a typical Indian wheat. The strong wheats selected were not of the same class. Pusa 4 is a large grained, amber wheat with strong straw, which comes to maturity with great rapidity. It is likely to prove of use in those tracts of India where the crop has to be grown on the minimum amount of water. It is also being tried in Bihar as a cover crop for Java indigo, the two crops being sown together as in the case of clover and barley in Europe. Pusa 8 is also an early white wheat with good straw, but the sample is mellow rather than translucent. Pusa 12 is a high yielding, white wheat with a large almost translucent grain, but this line ripens much later than the above and also tillers more. Pusa 22 is a distinctly hard translucent amber coloured wheat of Fife shape, but the straw is not strong enough to support a really heavy crop. Thus of the four strong wheats two, Nos. 4 and 22, are translucent with a dark amber tint, while No. 8 is distinctly mellow. No. 12 is intermediate between No. 8 and the two translucent lines.

The stations at which these wheats were grown were the same as in 1910 except that Raipur was substituted for Hoshangabad.

Trial of Muzaffarnagar white at nine stations, 1910-11.

	CAWNPORE.	PUSA.	ALIGARH.	BANKIPORE.	LYALPUR.	MEERUT.	ORAI.	PARTABGARH.	DUMRAON.
1. Type of soil	Heavy loam.	Light loam.	Loam.	Stiff clay.	Sandy loam.	Heavy loam.	Typical wheat soil (<i>mar</i>).	Loam.	Sandy loam.
2. Previous crop	Cotton.	Wheat.	Cotton.	As reaped paddy on 29th Sep. 1910.	Sown ploughed in.	Peas.	Gram.	Wheat.	Wheat.
3. Manuring	Nil.	Nil.	63 mds. pondrette per acre.	Nil.	Sown ploughed in on 1st Oct. 1910.	Nil.	Nil.	Nil.	83 mds. of cow-dung applied on 20th Oct. 1910.
4. Preparation for wheat	Ploughed six times with iron ploughs (14th March, 9th July, 3rd & 31st Aug., 19th Sep. & 13th Oct. 1910). Once with country plough (18th Oct. 1910).	Exposed several times by iron ploughs during previous hot season, afterwards fallowed.	Ploughed three times with iron ploughs (15th Jan., 6th Aug. & 21st Sep. 1910), and five times with the country plough (28th July, 30th Sep., 11th, 24th, 27th and 30th Oct. 1910).	Ploughed eight times between 4th & 6th Nov. 1910.	Harrowed on 29th Oct. 1910 and irrigated on 5th Nov. 1910, previous to sowing. Ploughed on 16th Nov. 1910.	Seven ploughings with the country plough (20th July, 4th & 16th Aug., 25th Sep., 18th, 23rd & 25th Oct. 1910).	Ploughed once with iron plough on 21st July 1910, and cultivated once with the <i>bakhar</i> on 16th Oct. 1910.	Ploughed five times with iron ploughs (7th & 11th April, 5th May & 6th June, 7th July 1910), and five times with the country plough (16th July, 13th, 18th, 22nd & 23rd Oct. 1910).	Ploughed eight times between 7th June 1910 and 21st Oct. 1910.
5. Date and method of sowing	28th Oct. 1910, behind the country plough.	22nd Oct. 1910, behind the country plough.	5th Nov. 1910, drilled.	8th Nov. 1910, with local drill.	12th Nov. 1910, behind the country plough.	2nd Nov. 1910, behind the country plough.	6th Nov. 1910, behind the country drill.	24th Oct. 1910, behind the country plough.	30th Oct. 1910, with local drill.
6. Seed rate (lbs. per acre)	60	55	82	100	60	100	100	120	100
7. Rain. { April 1st to fall in- sowing time inches { During growth period	26.54 1.87	32.11 1.42	26.30 2.57	55.59 1.03	15.52 4.05	29.23 5.52	30.50 2.58	45.21 4.33	48.96 3.42
8. Irrigation	Once (12th Nov. 1910).	Nil.	Three times (5th Dec. 1910, 9th & 27th Feb. 1911).	Once on 19th Jan. 1911.	Four times (22nd Dec. 1910, 16th Feb., 8th March & 13th April 1911).	Once on 3rd Dec. 1910.	Once on 21st Dec. 1910.	Twice (8th Dec. 1910 & 11th Feb. 1911).	Twice (12th Dec. 1910 & 12th Feb. 1911).
9. Date of harvesting	29th March 1911.	27th March 1911.	12th April 1911.	4th April 1911.	22nd April 1911.	14th April 1911.	5th April 1911.	22nd March 1911.	1st April 1911.
10. Growth period in days	151	156	158	147	161	163	151	148	153
11. Consist. { % Soft ency of { % Inter- sample { mediate { % Hard	3 58 39	01 72 27	42 45 13	60 35 5	85 15 0	15 65 20	44 49 7	27 61 12	2 8
12. Nitrogen percentage	1.93	2.05	1.34	1.46	1.43	1.75	1.44	1.43	1.65
13. Weight of 1,000 grains in grammes	34.18	33.03	28.84	35.51	36.15	30.52	31.18	34.18	29.05
14. Order of merit in commercial value	First.	Second.	Third.	Fourth.	Fifth.	Not tested.	Not tested.	Not tested.	Not tested.

Trial of Pusa 8 at nine stations, 1910-11.

	CANWARP.	PUSA.	ALGARH.	BANKIPUR.	LYALLPUR.	MEERUT.	ORAI.	PARTHARGH.	DUMRAON.
1. Type of soil	Heavy loam.	Light loam.	Loam.	Stiff clay.	Sandy loam.	Heavy loam.	Typical wheat soil (<i>mar</i>).	Loam.	Sandy loam.
2. Previous crop	Cotton.	Wheat.	Cotton.	<i>Ans</i> paddy harvested on 29th Sep. 1910.	<i>San</i> ploughed in.	Peas.	Gram.	Wheat.	<i>Ans</i> paddy removed on 1st Oct. 1910.
3. Manuring	Nil.	Nil.	63 mds. poudrette per acre.	Nil.	<i>San</i> ploughed in on 1st Oct. 1910.	Nil.	Nil.	Nil.	83 mds. cow-dung per acre applied on 22nd Oct. 1910.
4. Preparation for wheat	Six ploughings with iron ploughs (16th March, 9th July, 3rd & 31st Aug. 1910, 19th Sep. & 13th Oct. 1910). Once with the country plough (18th Oct. 1910).	Exposed several times by iron ploughs during previous hot season, afterwards fallowed.	Five ploughings with iron ploughs (10th June, 5th & 27th Aug., 5th & 22nd Sept. 1910). Six ploughings with country ploughs (27th July, 28th Sept., 12th, 24th, 27th & 30th Oct. 1910).	Ploughed eight times with iron ploughs between 4th and 6th Nov. 1910.	Harrowed on 29th Oct. 1910 and irrigated on 5th Nov. 1910, previous to sowing. Ploughed on 10th Nov. 1910.	Seven ploughings with country plough (20th July, 4th & 16th Aug., 25th Sep., 18th, 23rd, 25th Oct. 1910).	Ploughed once with iron plough (21st July 1910), and sowed once with <i>bakhar</i> (16th Oct. 1910).	Five ploughings with iron ploughs (7th & 11th April, 5th May, 6th June & 7th July 1910), and five ploughings with country plough (16th July, 13th, 18th, 22nd and 23rd Oct. 1910).	Ploughed once with iron plough (10th Oct. 1910) and four times with country plough a fortnight afterwards.
5. Date and method of sowing	28th Oct. 1910, behind the country plough.	22nd Oct. 1910, behind the country plough.	4th Nov. 1910, with local drill.	8th Nov. 1910, with local drill.	12th Nov. 1910, behind the country plough.	2nd Nov. 1910, behind the country plough.	6th Nov. 1910, with local drill.	24th Oct. 1910, behind the country plough.	30th Oct. 1910, behind the local drill.
6. Seed rate (lbs. per acre)	60	55	82	100	60	100	100	120	100
7. Rain-fall in inches { April 1st to sowing time During growth period	26.54 1.87	32.11 1.42	26.30 2.57	55.59 1.63	15.52 4.65	29.23 5.52	36.50 2.58	45.21 4.33	48.96 3.42
8. Irrigation	Twice (20th Nov. & 4th Feb. 1911).	Nil.	Three times (4th Dec. 1910, 9th Feb., 26th Feb. 1911).	Once (19th Jan. 1911).	Four times (22nd Nov. 1910, 16th Feb., 8th March & 13th April 1911).	Once (3rd Dec. 1910).	Once (21st Dec. 1910).	Twice (8th Dec. 1910 & 11th Feb. 1911).	Twice (17th Dec. 1910, & 13th Feb. 1911).
9. Date of harvesting	18th March 1911.	18th March 1911.	30th March 1911.	17th March 1911.	19th April 1911.	14th April 1911.	27th March 1911.	10th March 1911.	14th March 1911.
10. Growth period in days	140	147	145	129	158	163	142	136	135
11. Consistency of sample { % Soft % Intermediate % Hard	3 56 41	7 65 28	27 64 9	62 33 5	83 17 0	57 26 17	46 37 17	57 32 11	90 9 1
12. Nitrogen percentage	2.22	2.41	1.46	1.86	1.57	1.76	1.80	1.54	1.78
13. Weight of 1,000 grains in grammes	41.75	43.75	38.87	40.60]	34.02	37.41	39.40	38.88	42.27
14. Order of merit in commercial value	First.	Second.	Third.	Fourth.	Fifth.	Not tested.	Not tested.	Not tested.	Not tested.

Trial of Pusa 12 at nine stations, 1910-11.

	CAWNPORE.	PUSA.	ALIGARH.	RANKPORE.	LYALLPUR.	MEERUT.	ORAI.	FATEHABADH.	DUMRAON.
1. Type of soil	Heavy loam.	Light loam.	Loam.	Stiff clay.	Sandy clay.	Heavy loam.	Typical wheat soil (<i>mar</i>).	Loam.	Loam.
2. Previous crop	Cotton.	Wheat.	Cotton.	Ass paddy reaped on 29th Sep. 1910.	Ass ploughed in.	Peas.	Gram.	Wheat.	Ass paddy harvested on 1st Oct. 1910.
3. Manuring	Nil.	Nil.	63 mds. poudrette per acre.	Nil.	Ass ploughed in on 1st Oct. 1910.	Nil.	Nil.	Nil.	83 mds. cow-dung applied on 20th Oct. 1910.
4. Preparation for wheat	Six ploughings with iron ploughs (14th March, 6th July, 3rd & 31st Aug., 19th Sep., 13th Oct. 1910). One ploughing with country plough (18th Oct. 1910).	Exposed several times by iron ploughs during previous hot season, afterwards fallowed.	Six ploughings with iron ploughs (8th Jan., 5th July, 27th Aug., 5th, 18th Sep. & 26th Oct. 1910). Four ploughings with country plough (28th July, 12th Oct., 24th Oct. & 30th Oct. 1910).	Eight ploughings between 4th & 6th Nov. 1910.	Harrowed on 26th Oct. 1910 and irrigated on 5th Nov. 1910 previous to sowing. Ploughed on 10th Nov. 1910.	Seven ploughings with the country plough (20th July, 4th & 16th Aug., 23rd Sept., 18th, 23rd & 25th Oct. 1910).	Ploughed once with iron plough on 21st July 1910 and cultivated once with the <i>bakhar</i> on 16th Oct. 1910.	Ploughed five times with iron ploughs (7th & 11th April, 5th May, 6th June, 7th July 1910), and five times with the country plough (16th July, 13th, 18th, 22nd & 23rd Oct. 1910).	Ploughed once with iron plough on 10th Oct. 1910, four times with native plough (24th & 25th Oct. 1910).
5. Date and method of sowing	28th Oct. 1910, behind the country plough.	22nd Oct. 1910, behind the country plough.	5th Nov. 1910, with drill.	5th Nov. 1910, with local drill.	12th Nov. 1910, behind the country plough.	2nd Nov. 1910, behind the country plough.	6th Nov. 1910, behind the country drill.	24th Oct. 1910, behind the country plough.	30th Oct. 1910, with local drill.
6. Seed rate (lbs. per acre)	60	55	82	100	60	100	100	120	100
7. Rain-fall in inches.	26.54	32.11	26.30	55.59	15.52	29.23	36.50	45.21	48.96
8. Irrigation	Twice (20th Nov. 1910 & 21st Jan. 1911).	Nil.	Three times (4th Dec. 1910, 9th & 26th Feb. 1911).	Once on 19th Jan. 1911.	Four times (22nd Dec. 1910, 16th Feb., 8th March & 13th April 1911).	Once on 3rd Dec. 1910.	Once on 21st Dec. 1910.	Twice (8th Dec. 1910 & 11th Feb. 1911).	Twice (17th Dec. 1910 & 13th Feb. 1911).
9. Date of harvesting	24th March 1911.	23rd March 1911.	30th March 1911.	3rd April 1911.	19th April 1911.	14th April 1911.	27th March. 1911.	22nd March 1911.	2nd April 1911.
10. Growth period in days.	146	152	144	146	158	163	142	148	154
11. Consistency of sample	0 47 53	3 80 17	4 69 27	43 46 11	88 12 0	7 92 31	42 28 1.51	55 27 1.56	86 13 1.75
12. Nitrogen percentage	1.38	2.25	2.17	1.75	1.61	35.03	38.08	38.03	38.15
13. Weight of 1,000 grains in grammes	40.81	42.64	37.25	40.78	35.53	35.03	38.08	38.03	38.15
14. Order of merit in commercial value	First.	Second.	Third.	Fourth.	Fifth.	Not tested.	Not tested.	Not tested.	Not tested.

Trial of Pusa 22 at nine stations, 1910-11.

	Cawnpore.	Pusa.	Aligarh.	Bankipore.	Lyallpur.	Meerut.	Orat.	Partabgarh.	Dumraon.
1. Type of soil	Heavy loam.	Light loam.	Loam.	Stiff clay.	Sandy loam.	Heavy loam.	Typical wheat soil (mar).	Loam.	Loam.
2. Previous crop	Wheat.	Wheat.	Cotton.	<i>Aus</i> paddy reaped on 20th Sep. 1910.	<i>San</i> ploughed in.	Peas.	Gram.	Wheat.	<i>Aus</i> paddy harvested on 1st October 1910.
3. Manuring	Nil.	Nil.	63 mds. poudrette per acre.	Nil.	<i>San</i> ploughed in on 1st October 1910.	Nil.	Nil.	Nil.	83 mds. of cow-dung applied on 22nd Oct. 1910.
4. Preparation for wheat	Six ploughings with iron plough (5th May, 10th July, 4th Aug., 1st & 20th Sept., 14th Oct. 1910). One with country plough (18th Oct. 1910).	Exposed by iron ploughs during previous hot season, afterwards followed.	Five ploughings with iron plough (17th Dec. 1909, 6th Jan., 5th Aug., 5th & 17th Sep. 1910). Four ploughings with country plough (28th July, 12th, 24th & 30th Oct. 1910).	Eight ploughings between 4th and 6th Nov. 1910.	Harrowed on 29th Oct. and irrigated on 5th Nov. 1910 previous to sowing. Ploughed on 10th Nov. 1910.	Seven ploughings with country plough (20th July, 4th & 16th Aug., 25th Sep., 18th, 23rd & 25th Oct. 1910).	Ploughed once with iron plough on 21st July 1910 and cultivated once with the <i>batla</i> on 16th Oct. 1910.	Five ploughings with iron plough (7th & 11th April, 5th May, 6th June, & 7th July 1910), five ploughings with country plough (16th July, 13th, 18th, 22nd & 23rd Oct. 1910).	One ploughing with iron plough (10th Oct. 1910), four ploughings with country plough on 24th and 25th Oct. 1910.
5. Date and method of sowing	30th Oct. 1910, behind the country plough.	28th Oct. 1910, behind the country plough.	5th Nov. 1910, with drill.	8th Nov. 1910, with local drill.	12th Nov. 1910, behind the country plough.	2nd Nov. 1910, behind the country plough.	6th Nov. 1910, behind the country plough.	24th Oct. 1910, behind the country plough.	30th Oct. 1910, with local drill.
6. Seed rate (lbs. per acre)	60	55	82	100	60	100	100	120	100
7. Rain: (April 1st to fall in inches) (sowing time) (During growth period)	26.54 1.87	32.11 1.42	26.30 2.57	55.59 1.03	15.52 4.05	29.23 5.52	36.50 2.58	45.21 4.33	48.96 3.42
8. Irrigation	Three times (21st Nov., 17th Dec. 1910, 5th February 1911).	Nil.	Three times (9th Dec. 1910, 9th & 26th Feb. 1911).	Once (19th Jan. 1911).	Four times (22nd Nov. 1910, 16th Feb., 8th March, and 13th April 1911).	Once (3rd Dec. 1910).	Once (21st Dec. 1910).	Twice (8th Dec. 1910, 11th Feb. 1911).	Twice (17th Dec. 1910, 13th Feb. 1911).
9. Date of harvesting	26th March 1911.	26th March 1911.	31st March 1911	5th April 1911	29th April 1911.	14th April 1911.	5th April 1911.	22nd March 1911.	2nd April 1911.
10. Growth period in days	148	149	145	148	159	163	151	148	154
11. Consistency of sample (Soft ..) (Intermediate) (Hard ..)	0 25 75	5 34 61	3 45 52	26 53 21	91 9 0	5 30 65	1 33 66	53 29 18	53 34 13
12. Nitrogen percentage	1.91	1.97	1.55	1.70	1.74	1.79	1.83	1.63	1.68
13. Weight of 1,000 grains in grammes	34.80	30.12	28.58	32.20	25.42	25.58	26.25	29.70	26.07
14. Order of merit in commercial value	First.	Second.	Third.	Fourth.	Fifth.	Not tested.	Not tested.	Not tested.	Not tested.

Raipur is situated in the Chattisgarh Division of the Central Provinces and is characteristic of the black, moisture-retaining soils of this part of India. The three most important stations of North-Western India are Cawnpore, Aligarh and Lyallpur. At all these the crop is grown by means of canal irrigation, and all are situated in tracts, which, at the present time, grow large quantities of weak wheats like Muzaffarnagar for the export trade. It is sometimes stated that high quality wheats cannot be grown under canal irrigation in Northern India so that the results of the present trials are of particular interest.

The cultivation details relating to these various samples are given in the tables, and the results of the milling and baking tests are dealt with in full below. As the cost of milling and baking the whole thirty-six samples would have been very great, a selection had to be made, and for this purpose Cawnpore, Pusa, Aligarh, Bankipore, Raipur and Lyallpur were chosen.

REPORT BY MR. A. E. HUMPHRIES (PAST PRESIDENT OF THE NATIONAL ASSOCIATION OF BRITISH AND IRISH MILLERS) ON TWENTY-FOUR SAMPLES OF INDIAN WHEAT, GROWN IN 1911.

“ In each of the last three years, I have tested and furnished a report upon various sample lots of Indian wheat sent to me by the Indian Government for that purpose. In those reports, I have discussed many points concerning the qualities and commercial value of wheat and wheaten flour, which I need not repeat at length herein. It may, however, be desirable to summarise a few of them.

By the term strength I mean the capacity for making large, shapely, well aerated loaves, by stability the facility with which large masses of dough can be handled in the bakehouse. Good flavour implies a pleasant moistness and slight sweetness in bread at least one day old. Good colour means brightness of appearance, associated either with whiteness or with a slight yellowness. Dinginess is bad whatever the hue may be, and yellowness must be slight, if the flour or bread is to be highly esteemed for colour. The capacity for making a large quantity of bread from

a given quantity of flour is another unit of quality, separate and distinct from strength or stability, and having regard to the fact that in all these tests, I am dealing with small quantities of wheat, I have made no attempt to determine the relative merits of these sample lots on this point.

The colour of the bran does not necessarily indicate the colour of the endosperm; wheat with a red skin may yield a very white flour, or a wheat with a white skin may yield a very yellow flour. Nor is a red wheat necessarily or even probably stronger than a white one; but inasmuch as white bran is generally more valuable as a commercial commodity than red bran, also because powdered white bran is less discolouring than powdered red bran, and as in grinding, some of the husk always is powdered, white wheat is better than red wheat, if on all other points they are actually or approximately equal. But on the other hand, the growing of red wheat need not be discouraged if there be any valid general or local reasons for preferring some existing red varieties, or if there should be any prejudice in some parts of India in favour of red wheat.

British millers do not object to hardness in ordinary wheat (*T. vulgare*) nor do they object to mellowness, so long as the wheat is free-milling. They very strongly prefer wheat which can be easily separated into its commercial constituents, so that the bran can be removed with a minimum of grinding, and the flour be separated with a minimum of trouble in dressing.

Relative commercial values depend upon supply and demand and strong wheats are ordinarily in relatively small supply and command higher prices than weak wheats. But there will always be a demand for some weak wheats, from which flours most suitable for the manufacture of biscuits or puddings can be produced, or which can be mixed with very strong wheats for the production of many typical British bread flours. From these premises it follows that the difference in commercial value between strong and weak wheats is not a constant one, but does and will vary from time to time as the relative supply of each type varies. On the average

of seasons, however, strong wheats are likely to command substantially higher prices than weak ones.

All Indian wheats possess stability in a high degree, that is to say, the baker handling large masses of dough produced from flour obtained from Indian wheats only, is not at all troubled by stickiness or kindred faults, but some of the new varieties possess in addition to that good point, the toughness and resilience of dough characteristic of American or Canadian Spring wheats. This improvement and increase in strength as herein defined, can be developed by skilful wheat conditioning and by the use in baking of malt extracts possessing high diastatic power. Some wheats possess potentialities which can be developed in this way, others do not.

I have received for testing this year five varieties of wheat:—

Muzaffarnagar.

Pusa 8.

Pusa 12.

Pusa 22.

Pusa 4.

Each of the first four varieties was grown at five places—

Aligarh.

Bankipore.

Cawnpore.

Lyallpur.

Pusa.

One of the varieties (Pusa 22) was also grown at Pusa on land which had been waterlogged. Of these four varieties, I therefore received twenty-one lots. I also received three lots of the variety Pusa 4; one grown at Pusa, another at Raipur on unirrigated land and another at Raipur on irrigated land. All of these twenty-four sample lots were white wheat.

I have cleaned, conditioned, milled and baked each one separately. It was necessary to clean them, because the samples from Lyallpur, and in lesser degree the samples from Pusa were dirty. They appeared to have been subjected to unfavourable

climatic conditions during harvesting or threshing. However, I was able to remove that blemish by washing and scouring.

Investigations I have made in the last twelve months and recorded at the Portsmouth Meeting of the British Association, show that wheat conditioning brings about small but fundamental changes in the constitution of the flour. I have, therefore, amplified my usual programme for conditioning such sample lots, and have sought in still greater degree to give each lot the degree of conditioning it required. With that object, I conditioned them all slightly at the outset and then increased the percentage of added water in those cases which would be improved by the addition. As a result of this part of the investigation, the Muzaffarnagars and Pusa 8 received much less conditioning water than the others. A very great, indeed I may say an extraordinarily great improvement was brought about in Pusa 22 in this way, and substantial improvements were made in Pusa 12 and Pusa 4.

I baked almost all of them four times each. For the first two trials in each case, I used flour, water, salt and yeast only. Almost invariably Indian wheat is mixed by British millers with other kinds, so in the second round of baking trials, I used a typical British flour milled from a mixture of several wheats, foreign and English, taking in each case 50% of that flour and adding 50% from each sample lot of Indian. For the third round, I used a very small percentage of malt extract and grouped the flours according to the place where the wheats had been grown. For the fourth round, I again used the malt extract and reverted to the grouping of flours according to the variety of the wheats.

Following my usual custom, I examined each sample by eye before milling: a method which is, of course, used by millers in buying wheat, especially the first lot of any new kind. Having finally written down all the data upon which I should form opinions, I then referred to previous reports to see in what way results had been affected by seasonal, or agricultural causes in India, or any other way.

Muzaffarnagars.

All these five lots are typical of the variety, and I need not describe them in detail. In previous years, I have received among a larger number of Muzaffarnagar samples, consignments raised at the five centres from which this year's sample lots came. Judged by appearance and aided by reference to former reports I find the following interesting comparisons can be made concerning the order of merit in each season:—

1909.	1910.	1911.
Cawnpore.	Pusa.	Cawnpore.
Pusa.	Cawnpore.	Pusa.
Aligarh.	Aligarh.	Aligarh.
Bankipore.	Bankipore.	Bankipore.
Lyallpur.	Lyallpur.	Lyallpur.

In 1909, the samples represented great differences in money value. In 1910, those differences were much smaller. This year they are again relatively small. The Cawnpore lot is of very pleasing appearance, very good indeed of its kind. Pusa is not much behind it in appearance. The Lyallpur lot was dirty, but apart from that unfortunate and presumably avoidable blemish it is substantially as good in appearance as the Aligarh and Bankipore lots. This variety should not be heavily conditioned, and if that precaution be observed, it can be milled satisfactorily. There was no appreciable difference between the five lots concerning that stage of my investigation. Judged by their behaviour in the bakehouse, the differences are substantial but not great. Cawnpore and Pusa are very close together at the top, Bankipore and Lyallpur at the bottom, so that their relative values, judged by appearance, are a correct index of their baking values.

The Lyallpur loaves possessed a crust characteristic of Rivet wheat, one of our commonest English varieties, and more nearly resembled the ordinary Kurrachee wheats of commerce than any other sample. From this remark it may be inferred that the other four lots are superior in baking value to ordinary Kurrachee wheat.

The presence of translucent berries improves the appearance of a sample according to usual ideas concerning commercial excellence, but that causes the flour obtained from such lots to be brighter and more yellow, or at least less white than that produced from lots of the same variety which contain no translucent berries. So whereas the bread produced from this Lyallpur lot was worse in colour and general appearance than the others, the flour itself was relatively white in hue, and therefore, quite as well fitted for the making of puddings and biscuits as the substantially better lots from Cawnpore and Pusa.

I need not refer in detail to my previously expressed opinions concerning this variety. For certain purposes and under certain conditions it yields good flour, but for modern requirements of actual milling and for the making of bread, either of the other varieties sent me this year are much better having regard to their behaviour at all five places.

Pusa 8.

In some respects this wheat is not unlike Muzaffarnagar, and the similarity is more apparent in the lots grown at Bankipore and Aligarh than in others. The Lyallpur lot has a dusty, dingy appearance and has a black spot at the germ end of the berry. This last named blemish occurs also in the Pusa sample, and to a very slight extent in that grown at Cawnpore. However, that does not seem to damage the flour and need not be regarded as a serious fault. The Cawnpore has the appearance of strength and so has the Pusa lot, but in lesser degree. The others look weak, that from Lyallpur decidedly so. According to appearance the five lots should be placed in the following order of merit:—

Cawnpore.

Pusa.

Aligarh.

Bankipore.

Lyallpur.

In milling, this variety behaves similarly to Muzaffarnagar, possessing as it does the same typical mellowness, so that for optimum results it does not require and will not satisfactorily respond to much conditioning. But there the similarity ends so far as my work is concerned. In the bakehouse the Pusa and Cawnpore lots show a most decided superiority to those from Aligarh, Bankipore and Lyallpur. Both yield tough doughs, the Pusa lot particularly, and from both lots fine large loaves can be obtained. There is a superiority in the other three lots over the Muzaffarnagar grown at the same places, but they all yield the same type of flour. When by lapse of time the conditioning had worked its full effect and when malt extract was added in baking, the Pusa lot behaved very much like Russian Ghirka wheat, and this of itself is noteworthy. The bakehouse results, in the case of this variety also, leave the order of merit unchanged from that assigned to them by their appearance, but the Pusa and Cawnpore lots are very close together, and at a considerable distance the other three lots are also close together.

Pusa 12.

This variety also resembles Muzaffarnagar in appearance, and it is free from the black spot at the germ end of the berry which is seen in some samples of Pusa 8. It can be described as a mellow wheat, but it differs from Muzaffarnagar and in lesser degree from Pusa 8, because its baking qualities are improved, and its behaviour in the milling processes is not prejudicially affected by the addition of water in conditioning, even if that process be applied severely. The five samples, judged by appearance, are in the following order of merit:—

Cawnpore.
Pusa.
Aligarh.
Bankipore.
Lyallpur.

and the differences between them are great, for the Cawnpore lot is a very beautiful sample, and the Lyallpur a distinctly poor one. Stated in money value, the difference is from half a crown to three shillings per quarter. The Pusa sample is a good one, and so are the Aligarh and the Bankipore lots, but in the cases of this variety, the differences between each lot are approximately equal, the best being very good, the worst distinctly poor. I find, however, this important difference in baking value. In the cases of Muzaffarnagar and Pusa 8 already dealt with, the differences disclosed by baking tests are substantial. In this case, the baking results tally in order of merit with my judgment as to appearance, but the differences are not large. This really means that the Aligarh, Bankipore and Lyallpur lots bake out really well. From the last named, we did not obtain a tough dough, but in increasing degrees from all the others we do so, and a baker who has to handle large masses of dough regularly for stability would very much prefer Pusa 12 flour to Muzaffarnagar and in lesser degree to Pusa 8.

Pusa 22.

This variety differs greatly on several points from Muzaffarnagar, Pusa 8, and Pusa 12. The berries are comparatively short and would be described in technical phrase as round or short-berried, whereas the others would be described as long-berried. It is distinctly hard by nature whereas the others are mellow. I have not hereinbefore used the terms woolly and its technical opposite free-milling, which I explained at length in previous reports, but I may say at this stage that whereas Muzaffarnagar and Pusa 8 are mellow and either slightly or markedly woolly in texture (in other words possess physical characteristics which prevent the miller from making separations of branny husk from kernel easily), the Pusa 12 is mellow and free-milling; and Pusa 22 is hard and free-milling. Furthermore, the addition of water in conditioning to Pusa 12 improved its baking qualities and was not required to improve its behaviour in the milling processes; but in the case of Pusa 22, the conditioning was desir-

able for both reasons and brought about a remarkable change. All these lots contained a comparatively low percentage of water when I made the first round of milling tests. When the flours obtained from that round were baked. Pusa 22 gave a surprising result, for I knew that it was White Jana Khar which, in 1909, yielded flour of the highest class, possessing among other first rate characteristics a really great strength; yet from the first milling this season we obtained flour which was not strong, though first rate in other respects. Finally, after some experimenting, I raised the water content of the wheat to high figures, and then let some part of it remain for a considerable time before it was milled. Then we obtained results in the bakehouse similar to those of 1909, and the behaviour of the variety during the milling processes of grinding and dressing was first rate. Even so, I have not this year obtained quite so good baking results as in 1909, but the miller and baker would be extremely hard to please if they were not content with them even this year. Judged by appearance, we again get the same order of merit as in the previous cases stated herein—

Cawnpore.

Pusa.

Aligarh.

Bankipore.

Lyallpur.

with this difference, that the Pusa and Aligarh lots should be bracketed equal. Furthermore, the appearance of these wheats provided a forecast of some of the baking results already specified, for though the Cawnpore lot looked strong, the others did not this season appear to be nearly so strong as the one lot of this variety which I received in 1909. All of them are really good as regards stability, and the order of merit on that point of quality agrees with their appearance as recorded above. In that respect they stand well in competition with wheat from any part of the world and the Cawnpore lot gets into the very highest class. As regards the one point of strength (size of loaf) the results are not so good, and all five lots

are close together, but taking all points of quality into consideration the results are very good indeed. The extra lot from Pusa, grown on land which had been waterlogged, contained a substantial proportion of shrivelled or imperfectly developed grain, and this would reduce its commercial value *6d.* to *9d.* per quarter below that of the Pusa lot grown under normal conditions. Apart from that there is practically no difference between the two samples. The preference would be given to the normally grown lot on the other points of quality, but it would be a preference only, representing no further difference in commercial value.

Pusa 4.

All three samples of this variety (Pusa, Raipur unirrigated and Raipur irrigated) are in the category of hard, free-milling wheats. The Raipur unirrigated lot is truly superb in appearance, indeed I do not think I am exaggerating if I say, it is the finest looking lot of Indian wheat I have ever seen. The Raipur irrigated is distinctly inferior to it in appearance, but is nevertheless a very beautiful sample. The lot from Pusa suffers by the comparison. It contains some damaged corns and is dingy in appearance. The black spot at the blunt or germ end of the berry occurs this year again in this lot, and it is a blemish, though not a serious one. Nevertheless this lot appears to be strong and is so, in fact as well in appearance. Unfortunately, I received only comparatively small quantities of this variety, so have not been able to make extended baking tests with it. But the results I did obtain were good. In hue, and general appearance of crust and crumb, the Raipur lots are of the very highest class, but on the one point of strength they are really good without being extraordinary judged by the standard of typical London flour. The Pusa lot is, however, extremely good on all points including strength, in other words, is better than it looks. A miller buying the wheats for the first time on appearance only would unhesitatingly place the Raipur unirrigated lot not only at the head of the Pusa 4 variety, but of the entire lot I have received this season; but having milled and baked them under the conditions

described herein, I should myself choose the Pusa 4 lot grown at Pusa for that position.

The Wheats grown at Pusa.

In previous years I have reported very favourably on wheats of the Pusa Nursery group, that is to say, on new varieties obtained by selection or hybridizing at Pusa. I had also noted that, as a rule, any variety grown at that centre behaved better than the same variety grown elsewhere. So I recommended that wheats of the Pusa Nursery group should be tried at several centres before a final opinion as to their degree of superiority should be given. I was, therefore, pleased to see that the Economic Botanists at Pusa and Cawnpore had by their action anticipated my recommendation, rendering it possible to see how some of the varieties behaved at several places in India. It is very well known that no variety is likely to suit all environments. This is abundantly evident in the United Kingdom, much more is it likely to be so in a country of great distances like India. I have, therefore, in dealing with this year's consignment of twenty-four samples examined them not only by groups according to variety, but also by groups according to their place of origin, and I desire to make the following remarks upon the last named classification. It will have been seen how extremely uniform the order of merit is this year in the case of all varieties. I have received no information directly or indirectly as to the climatic conditions prevailing this year at Pusa during the maturation, harvesting and threshing of its wheats, but I should suppose from the appearance of the samples that those climatic conditions must have been relatively unfavourable. However, as a miller with considerable experience, I am used to evidences of such mishaps and probably differ from the grower or merchant in appraising their importance. That is the reason why I have again placed the wheats grown at Pusa so highly according to appearance. But the baking results confirm my opinions based on appearance and I need at this juncture merely consider how, as regards quality, each of the five varieties suits the environment furnished by Pusa.

Judged by appearance the blemishes already mentioned, kept Pusa 4 from the first place. For that position Pusa 8 and Pusa 12 could be bracketed. Then I placed Pusa 4, with Pusa 22 and Muzaffarnagar following. But milling and baking trials upset that order, and in spite of its blemishes, Pusa 4 goes to the top and the order of merit is this :—

Pusa 4.
Pusa 8.
Pusa 12.
Pusa 22.
Muzaffarnagar.

Cawnpore Wheats.

I suppose the climatic conditions at this centre must have been excellent for all four varieties grown there are of beautiful quality and appearance. I received no sample of Pusa 4 from Cawnpore or the places remaining to be mentioned. On appearance the order of merit is simple, for I bracketed Pusa 12, Pusa 8 and Pusa 22 equal, then the Muzaffarnagar. The milling and baking tests, however, sort them out and the order of merit then is,—

Pusa 12.
Pusa 8.
Pusa 22.
Muzaffarnagar.

The Pusa 12 and Pusa 8 are distinctly superior to the other two varieties at this centre.

Aligarh Wheats.

In no case is the Aligarh sample equal either to the Cawnpore or Pusa lot, but appearances and the milling and baking tests agree in revealing substantial differences and the order of merit is—

Pusa 12 }
Pusa 22 } equal.
Pusa 8.
Muzaffarnagar.

In this case the Muzaffarnagar is not far below Pusa 8.

Bankipore Wheats.

In this case also appearances and the milling and baking results agree. The differences though appreciable are not so great as in the former cases, and each sample is pale in flour.

The order of merit is—

Pusa 12	} very nearly equal.
Pusa 22	
Pusa 8.	
Muzaffarnagar.	

Lyallpur Wheats.

Believing that this centre represents a district in which a large quantity of Muzaffarnagar is grown, I have been curious to see how the comparison would come out. When examining these wheats by appearance, I dictated the following note :—

“Muzaffarnagar is the best looking, the others are so dingy and generally poor, that on appearance nobody would give more for them. They may be slightly stronger, but from appearance Muzaffarnagar is the best wheat grown at this station, and there is practically nothing between the others.” I shall be glad to learn in due course whether the climatic conditions during harvesting or threshing were alike for all four varieties; whether for instance the Muzaffarnagar ripened earlier or later than the others, and so escaped some bad weather from which the others suffered; alternately whether they were threshed under comparable conditions. If the former alternative be the reason why the three new varieties appear to be relatively poor in appearance, it will be interesting to know whether the unfavourable conditions are likely to occur in other seasons, or whether they are merely an accident of the last one.

In the milling processes Muzaffarnagar is rather better than Pusa 8, but inferior to Pusa 12 and Pusa 22. In the bakehouse the effects of superior breed triumph, as they usually do, over other influences under such conditions as these, so on baking results the order of merit is this :—

Pusa 12.
Pusa 22.

Pusa 8.

Muzaffarnagar.

and the differences are substantial.

I believe that Sir James Wilson has advocated in public the separate marketing of Muzaffarnagar wheat, and I certainly endorse his recommendation and extend it. It can readily be understood that the shipping of great quantities of wheat from one port is facilitated by making up a commercial type upon which a very great business can be transacted, but, on the other hand, the later developments of milling have intensified the importance of treating each wheat according to its individual requirements. Choice white Kurrachee is a mixture of two principal types of wheat, one (principally Muzaffarnagar) mellow and woolly, the other hard and free-milling kinds. No miller can make the best of both kinds when he receives them mixed together. He may desire or be perfectly willing to buy both, but if sellers wish to make the most of such widely differing wheats, they must, by offering them separately, put the miller in a position to treat each one to the best advantage. I do not wish to advocate any extreme course, or I should say that it would be wise to keep Muzaffarnagar and Pusa 8 separate, if the latter should be grown to a substantial extent in the Punjab or United Provinces, but bearing in mind the exigencies of a large export business, I would as a practical proposition be content to see these two varieties grouped together for export, but if Pusa 12, or more emphatically if Pusa 22 be largely grown in districts supplying the port of Kurrachee, the recommendation to keep them separate from Muzaffarnagar is important.

Summary.

The results I have obtained again demonstrate the well-known fact that differences in soil, climate and water-supply do materially affect the quality of wheat. I need not emphasize that point now. But another point is equally apparent from these tests; that the inherent and hereditary influences of breed are extremely important,

perhaps predominant. The following table will illustrate the point omitting for this purpose Pusa 4 altogether :—

	<i>Pusa 12.</i>	<i>Pusa 22.</i>	<i>Pusa 8.</i>	<i>Muzaffarnagar.</i>
Aligarh ..	1	1	3	4
Bankipur ..	1	1	3	4
Cawnpore ..	1	3	2	4
Lyallpur ..	1	2	3	4
Pusa ..	1	3	1	4
	<hr/> 5	<hr/> 10	<hr/> 12	<hr/> 20

In this table I have set out the order of merit indicated by milling and baking tests, and the figures at the bottom of each column show how on results so obtained, Pusa 12 comes out top with Pusa 22 and Pusa 8 close together and Muzaffarnagar last. The additions are made merely to indicate the order of merit and not their relative degrees of excellence. For my own guidance, I have recorded results in marks and the addition of them, grouped according to variety, yields the same order of merit. I have already shown that the differences between the varieties are not the same at all centres, but the broad principle comes out clearly from these tests that the effect of breed is predominant or at least is extremely important. I do not think, however, that any one variety will be equally suitable for all those parts of India in which ordinary wheat (*T. vulgare*) is or can be grown, and I should like to see these tests continued and extended to other varieties.

I should also like to repeat the opinion expressed in my previous reports, that the financial return to the grower should be the predominant influence in determining the suitability of any or all varieties of wheat in India as elsewhere. British millers are likely to pay relatively high prices for the wheats I have tested, compared with the existing typical Kurrachee and Calcutta wheats exported, and this remark includes Muzaffarnagar also, for that can be used satisfactorily for special purposes, and is for them more valuable than ordinary Choice White Kurrachee, or No. 2 Club, Calcutta ;

but British millers are not likely to pay an increased price sufficient to compensate the grower for a substantially diminished yield. Furthermore, British millers are in a better position than ever they were to make the best use of any and every kind of wheat offered them, provided widely differing kinds are sold separately and not mixed together.

It seems to me, that this year's results are most encouraging to all concerned in the work. I have been compelled in handling small quantities of wheat to use methods of milling inferior to those used in ordinary milling plants worked commercially, but the bake-house results I have obtained are astonishingly good as regards the colour and general appearance of the bread obtained, and judged on all points of quality any one of these wheats, Muzaffarnagar included, provided the precautions I have specified are observed, are on the whole much superior to those obtained from ordinary Indian wheats milled on a commercial scale, and as we have seen, the newer varieties are superior for bread making to Muzaffarnagar. I should especially like to see Pusa 4 tested extensively, and the cause for its small black spot blemish investigated. On balance of points, I selected it from last year's set of samples for special comparison with Manitoba wheat, and most certainly it is a variety which should be carefully watched and tested as one of several likely to be very useful in providing first class and much improved wheat for export to Great Britain."

WEYBRIDGE, ENGLAND. }
The 29th November 1911. }

A. E. HUMPHRIES.

Several very interesting results are to be found in this report which it is proposed to deal with briefly. The first relates to the tests of the Muzaffarnagar samples which are summed up in the table following (see page 87). These behaved in 1911 practically like those

Comparative value of *Muzaffarnagar* white grown at *Nine Stations in 1908-09, 1909-10 and 1910-11.*

Where grown.	Consistency.						Weight of 1,000 grains in grammes.			Nitrogen per centage.			BAKERS' MARKS.			Order of merit in commercial value.							
	1908-09.			1909-10.			1910-11.			1908-09.	1909-10.	1910-11.	Stability.	Strength.	Stability.	Strength.	1908-09.	1909-10.	1910-11.				
	Soft.	Inter.	Hard.	Soft.	Inter.	Hard.	Soft.	Inter.	Hard.														
Cawnpore	8	48	39	5	72	23	3	58	39	35.69	38.07	34.18	2.37	1.79	1.93	84	75	72	62	First	Second
Pusa	16	59	25	0	57	43	0	72	27	38.35	39.80	33.03	2.00	2.19	2.05	84	75	80	72	First	Second
Partabgarh	36	33	31	50	38	12	27	61	12	40.02	30.71	31.18	1.79	1.49	1.43	82	75	70	62	Third
Meerut	54	30	16	15	65	20	..	37.92	30.52	1.34	1.79	1.75	70	60	70	62	Sixth
Aligarh	76	15	9	57	35	8	42	45	13	42.54	34.58	28.84	1.93	1.90	1.34	70	60	70	62	Fifth
Orai	95	5	0	66	29	5	44	49	7	30.97	37.65	31.18	1.93	1.45	1.44	64	60	72	62	Sixth
Bankipore	83	13	4	79	14	7	60	35	5	38.95	42.58	35.51	1.34	1.48	1.46	68	52	72	62	Seventh
Dumraon	72	21	7	58	36	6	63	29	8	43.67	40.73	29.05	1.52	1.41	1.65	78	63	72	62	Fourth
Lyalpur	75	20	5	78	16	6	85	15	0	37.08	40.47	36.15	1.52	1.38	1.43	68	68	70	62	Eighth

In 1910-11 no bakers' marks were awarded and only five wheats were tested.

of the previous year, and it is clear that little or no further differences are likely to be obtained by the continued trial of this wheat. In the three seasons, 1909 to 1911, the order of merit in commercial value has been almost the same with Cawnpore and Pusa at the top followed by Aligarh and Bankipore with Lyallpur last. In no case has this wheat become strong, it has always remained a weak wheat with somewhat inferior milling qualities but when grown to perfection at Cawnpore and Pusa the sample has been improved in appearance, and has done better in the mill and bakehouse. This indicates, as would be expected, that in the maintenance of quality the methods of growing wheat are of some importance. As regards further work with this wheat it is proposed in 1912-13 to grow all the Muzaffarnagars from each centre side by side at Aligarh and to compare the resulting samples. It is probable that the environmental differences will disappear and that the seed from all the plots will be identical.

The chief result contained in the report relates to the behaviour of the strong wheats at the various centres and it is clear that no matter how unfavourable the conditions were under which the wheats were grown quality has not been lost. The results confirm those of Humphries and Biffen in England.¹ These investigators grew two wheats, differing in strength, on seven types of soil and found that while the soil had a considerable influence on the strength yet on all soils the stronger variety, Red Lammas, gave the better result. The results also confirm the English experience with Fife and its hybrids which yield strong flour. Fife wheat has retained its strength and high qualities even in England where the great majority of the wheats now grown are exceedingly weak.

In considering the results obtained with these strong wheats at the various stations in the plains the behaviour of Muzaffarnagar at Pusa and Cawnpore must be borne in mind. At these centres during the past few years a considerable amount of attention has been paid to the manner of growing wheat and to the conditions

¹ Humphries & Biffen, *l. c.*

under which optimum results, both as regards yield and quality, can be obtained. Under these conditions Muzaffarnagar has improved in quality. In the case of the strong wheats the best results were also obtained at Cawnpore and Pusa after which those at Aligarh follow in order of merit. At Bankipore the wheats were grown after rice the same year with a hurried preparation for the wheat crop. As will be evident from the results described in the next section of this paper such a preparation for wheat was the worst that could be devised. In spite of this, however, the strong wheats did not lose their qualities. At Lyallpur the preparation was distinctly inferior to that at Cawnpore and in addition the crop was watered four times. On the alluvium this kind of preparation and treatment of the crop is very detrimental and optimum results are not possible in this way. Even at this centre the milling and baking qualities asserted themselves and the strong wheats did best. Had the preparation at Bankipore and Lyallpur been equal to that at the other stations it is certain that the samples would have given still better results. To sum up the report, Pusa 12 proved the strongest wheat and did best at all stations producing good loaves even under adverse conditions. This wheat, in addition to its good quality is also a heavy yielder which is interesting in view of the statements often made that yield and quality in the same wheat cannot be combined. As regards stations, the best results were obtained at Cawnpore under canal irrigation, thus disposing of the idea that strength and high milling qualities are only possible under *barani* conditions at stations like Pusa.¹ The Cawnpore results are of particular importance in this matter as they prove that very fine samples of wheat of high quality and appearance can be grown under canal irrigation provided the cultivation is suitable and the amount of water is regulated. Similar samples were produced in 1910 and again in the present year, 1912.

The results obtained in 1911 with the Pusa samples were slightly inferior to those at Cawnpore. At the former station the climatic conditions in 1911 were relatively unfavourable. The soil moisture was deficient and rain, wind and fog, followed by hot winds just before harvest, did considerable damage to the crop.

At Raipur, the black soil station, the only strong wheat tested was Pusa 4. This was grown as a dry crop and also irrigated. The appearance of the Raipur samples was exceedingly fine and equal to any of the wheats that have ever been grown at Pusa. The grains were large with an absolute weight of over 45 grammes and in the milling and baking tests Mr. Humphries said: "In hue and general appearance of crust and crumb the Raipur lots are of the very highest class, but on the one point of strength they are really good without being extraordinarily judged by the standard of typical London flour." This is a most important result in a tract which produces so much weak soft wheat and it indicates that on the black cotton soils of the Peninsula wheats of considerably higher quality than those now cultivated can be grown to perfection. In the black soil tracts of the Central Provinces the yield of wheat is always low, anything over twelve bushels to the acre being very exceptional. The intractable nature of the soil and the difficulty in keeping the soil moisture near enough to the surface of the ground to promote tillering are adverse factors to any great increase in the yield. As is well-known the chief factor determining the yield of wheat is the amount of tillering. If the soil conditions are such that very little tillering can take place it is impossible to get heavy yields. Further, the shortness of the season is another factor operating in the same direction. To obtain even the present yields as much as 100 lbs. of seed to the acre has to be sown, so that the net increase per acre is not great and rarely exceeds 600 lbs. to the acre. In these tracts the plant breeder will find great difficulties in his path if yields like those easily possible in the plains are desired. With quality, however, the matter is quite different, and it is in this direction that the greatest chance of improvement lies. That this is a likely avenue of progress is proved by the Raipur results with Pusa 4 in 1911 which in addition to good quality also gave a very satisfactory yield. In 1912 still better results as regards yield with other high quality Pusa wheats were obtained at Raipur and Tharsa, the results of the milling and baking tests of which will be dealt with in a later paper.

From the breeding point of view the fact that strength can be maintained, both under canal irrigation in the alluvium and also on the black cotton soils of the Peninsula, is of the greatest importance. It opens up a valuable direction of improvement which offers unlimited scope after the maximum possible yield has been realised. Canada at the present time has reached a high position in the Home markets on account of the high quality of the spring wheats produced in Manitoba. Indian wheats, on the other hand, although drier and often better grown, nevertheless fetch a lower price solely because of the relatively poor quality of the flour they yield. Once high quality is introduced into Indian wheats they will be at least as valuable as any produced in the world. They are already very dry and give a high percentage of flour and with quality added would at least be equal to the best Canadian grades.

The importance of the behaviour of a wheat in milling, apart from the quality of the resulting flour, is very great. Millers prefer wheats which absorb a large amount of water in the conditioning process without losing the character of free milling or, in other words, the capacity of allowing an easy separation of bran and flour. Many of the present soft white wheats exported do not absorb a great amount of water and do not mill well so that in improving the wheats of India the purely milling aspect must be considered. It will be seen in the tests that in addition to strength the high milling qualities of the Pusa wheats were also maintained when grown at other stations. In the improvement of the wheats of any tract the milling aspect of the subject should receive careful attention.

IV. YIELD AND QUALITY IN WHEAT.

In the literature relating to yield and quality in wheat there appears to be a considerable confusion of ideas. As this is likely to stand in the way of progress an attempt has been made in this chapter to define the position in so far as it applies to India. There is a general opinion that in some manner yield and quality are antagonistic and that high yielding wheats are always of poor quality. On the other hand, if quality is aimed at, then the yields are necessarily poor. At a recent discussion on the improvement of English wheat at the Farmers' Club in London these erroneous ideas were advocated by Percival¹ who maintained that, under English conditions, yield and quality cannot be combined.

The results of our experiments indicate that there is a definite connection between yield and quality. These experiments can best be understood and their significance realised if the two aspects of the whole question are separately considered. In the first place, the experimental evidence on the possibility of combining high yield and high quality in the same wheat must be considered. The second point relates to the conditions under which, in any particular wheat, the best quality can be obtained.

The first aspect relates to the combination of yield and quality in the same wheat. On this subject there is a considerable volume of Indian evidence. At Pusa, several new hybrid wheats with high grain qualities, raised from Muzaffarnagar, have for several years given higher yields than either parent. These are Pusa 100, 101, and 106 which were tested by Mr. Humphries in 1910 and found to behave like Manitoban good grade wheats produced in a dry season.² Several other wheats from the same cross, which have

¹ *Journal of the Farmers' Club*, 1912, p. 80.

² Howard and Howard, *Bull. 22, Agricultural Research Institute, Pusa*, 1911, p. 14.

not yet received numbers, gave equally good results in 1912. In the trials of the strong wheats in 1911, Pusa 12 at most stations gave a higher yield than Muzaffarnagar, a result which is almost always the case when the two wheats are grown side by side at Pusa. In the case of gram (*Cicer arietinum*, L.) at Pusa, where many pure lines have been grown, the line with the highest quality is by far the highest yielder. Experience shows that there is no inherent antagonism between yield and quality and that both are possible in the same wheat.

Considerable attention has been paid at Pusa and afterwards at Cawnpore to the study of the conditions under which any particular wheat gives the best possible sample. As might have been expected the best samples were produced when the wheats gave the highest yield. The best samples were obtained after hot weather cultivation and clean fallowing during the monsoon when the objects aimed at were the absorption of water and its retention in the soil and subsoil combined with the destruction of all weeds. In this way yields of over 40 bushels to the acre have been obtained at Pusa without manure, without rain after sowing and without irrigation.

The higher the yield, the finer and more uniform the sample has been while the results of the milling and baking tests have always been most favourable in years of greatest yield. Thus in 1910 at Pusa, when the yields were the highest ever reached, the samples were particularly well spoken of by Mr. Humphries and gave very good results indeed when milled and baked. In 1911, the yields, due to very unfavourable weather, were lower, and in that year the samples were relatively poorer in appearance and the milling and baking results were also to a certain extent adversely affected. In the tests of Muzaffarnagar, grown at the various stations, a similar result has been obtained. The Cawnpore and Pusa samples have always done best in the milling and baking tests. At these centres this wheat has uniformly given higher yields than at the other stations. There is no doubt therefore that

in wheat growing the best sample is produced under those conditions which give the highest yield. This in reality clears up the whole matter as will be obvious from the experiments described below. These relate to hot weather cultivation and drainage—two important factors in wheat production in the alluvium.

A. HOT WEATHER CULTIVATION.

In previous papers¹ attention has been drawn to the marked effect of hot-weather cultivation in the production of wheat and other crops, both *kharif* and *rabi*, in the alluvium of the Indo-Gangetic plain. During the early period of the wheat experiments at Pusa, when attention was being paid to the best methods of growing the crop under Indian conditions, it was decided to try the effect of opening up the stubbles immediately after harvest and so exposing the soil to the hot dry winds which prevail at this period of the year. The stubbles were ploughed several times and thoroughly opened up resulting in the production of a deep dry mulch of fine soil in which no growth of weeds was possible. This enabled all the early monsoon rains to be absorbed, and the subsequent procedure consisted in sufficient cultivation to keep down weeds and to break up the surface so as to allow of the percolation of more water into the subsoil. In this manner sufficient moisture was absorbed for a wheat crop of over forty bushels to the acre and the fields rapidly became free of weeds. In the lighter lands, the water holding capacity of the soil was increased by ploughing in crops of *san* (*Crotalaria juncea*, L.) raised on the early monsoon showers, but this has not yet been found necessary in the heavier lands.²

¹ See *Nature*, Feb. 17th, 1910; *Memoirs of the Dept. of Agr. of India (Botanical Series)*, Vol. III, No. 4, 1910, and *Pusa Bulletin* No. 22, 1911.

² If green manuring with *san* is attempted on heavy wheat lands in Bihar, in years when these soils are waterlogged after the green crop is ploughed in, the resulting wheat crop is always less than if no manure had been added. The addition of the green crop seems to accentuate anaerobic fermentation in the soil and to reduce the available nitrogen for the wheat crop. Fortunately such heavy soils retain water well and are not in need of green manure for this purpose. The fact that green manuring these heavy lands for wheat reduces the yield seems to indicate that on rice lands in Bihar green manuring with *san* would increase the yield considerably.

The effect of hot weather cultivation and moisture conservation was then tried at Cawnpore and, as at Pusa, the effect was instantaneous. The detailed results are published elsewhere, and it is sufficient to say that crops of between 25 and 30 mds. to the acre of high quality wheat have been produced using half the quantity of irrigation water employed by the cultivators in the neighbourhood.

At this point it became desirable to determine the actual crop increase resulting from hot weather cultivation. It was unfortunate that both at Pusa and at Cawnpore all the land had been thoroughly cultivated in the hot season for at least two years before the experiment was started and none of the area had been left in its original condition. Under these circumstances it was expected that no great differences would be detected the first year, and that it would take some time for the fertility to fall to the ordinary level of that exhibited by the cultivators' fields.

The experiment at Pusa was commenced after the harvest of 1910, and a level plot of typical wheat loam of high moisture retaining capacity was selected for the purpose. One half was cultivated during the hot weather, the remainder being left untouched till after the beginning of the monsoon. Across both plots a strip of land was manured just before sowing with nitrate of soda at the rate of 224 lbs. per acre and the results are shown in the following plan :—

Ploughed after the beginning of the monsoon.	Ploughed from the hot season onwards.	
32.02	37.89	Unmanured.
35.72	37.52	Manured with nitrate of soda at the rate of 224 lbs. per acre.
32.03	37.89	Unmanured.

The numbers in the table are bushels per acre.

The figures show that late ploughing caused a fall in the crop of six bushels of wheat to the acre, and that the dressing of nitrate of soda partially made up for the deficiency on the late ploughed plot but added nothing to the yield of the early ploughed plot.

The consistency, absolute weight and nitrogen content of the samples in this experiment are given in the following table. Those manured with nitrate of soda were darker in tint than the others, while those from the late ploughed plot were comparatively pale in colour and not so well grown as the rest.

Treatment of the land.	Consistency.			Weight of 1,000 grains in grammes.	Nitrogen percentage.	Yield per acre.	
	Hard.	Intermediate.	Soft.			Mds.	Bushels.
Ploughed early.	88	12	0	32.29	2.19	27.62	37.89
Do. + 2 cwt. of nitrate of soda per acre.	83	12	0	31.48	2.57	27.35	37.52
Ploughed late.	71	29	0	30.91	2.28	23.35	32.02
Do. + 2 cwt. of nitrate of soda per acre.	81	19	0	31.11	2.48	26.05	35.72

The standard maund consists of 40 seers and is equivalent to 82.27 lbs.

In the following year, 1911-12, the experiment was repeated on the same plot, but in this case no nitrate of soda was applied. There was a distinct difference in vegetative vigour between the plots and this is reflected in the yield of grain as will be seen in the results obtained.

1. Early ploughing—35.41 bushels to the acre.
2. Late ploughing—22.90 bushels to the acre.

The difference in yield during the past year was twelve and-a-half bushels per acre, or about twice that obtained the first year of the experiment. The results indicate that the effect of hot weather cultivation is cumulative and that the effects are not lost for some

time. The experiment is being continued until the yield of the late ploughed plot becomes steady, after which it is proposed to reverse the treatment of the two plots.

As regards quality, the appearance of the wheat from the late ploughed plot was distinctly inferior to the other. It was paler in colour and not so well grown as that from the early ploughed plot. In this case, while the plot with the higher yield gave the better quality, the fall in yield was greater than the difference in quality. This agrees with our experience at Pusa in wheat growing that in the case of the same wheat any adverse condition always affects yield much more than quality. When quality is sensibly lost it is almost certain that the yield is poor.¹

B. DRAINAGE.

During the progress of the wheat investigations in India one important factor in the growth of this crop has frequently been observed. This is waterlogging both previous to and during the growth of the crop. If wheat lands in Bihar are inundated for any length of time during the monsoon, or, if portions of the fields are continuously waterlogged for long periods, then a sour or semi-marshy condition of the soil results which is shown by a yellow crop of poor vegetative vigour and low yield. Often the consistency of the resulting sample on such areas is affected and a large proportion of mottled and soft grains are produced, which spoil the appearance of the sample and lower its market value. Similar results are to be seen in canal irrigated tracts in lowlying areas of the fields which get too much water and in which the soil becomes semi-waterlogged for long periods. These effects were distinctly

¹In connection with these experiments the behaviour of the continuous wheat plot at Pusa may be of interest. This is a strip of typical wheat land which, for the past five years, has been cropped every year with wheat without manure. In 1911-12, the fifth year of the experiment, the yield per acre was 36.25 bushels, which is the highest yield of the variety (Pusa 22) so far obtained at Pusa. No diminution in vegetative vigour was observed. On the contrary, the growth was so great that a large portion of the crop was laid by wind soon after coming into ear which circumstance diminished the yield of grain. It will be interesting to see for how much longer these yields can be obtained and whether any organic matter besides the stubble need be added to the soil.

visible in the wheat plots at Bankipore and Dumraon in 1911 where wheat followed rice. The samples also contained a high percentage of soft and spotted grains. Drainage is therefore an important matter in wheat growing in India both as regards yield and quality even in areas where the crop is grown without any appreciable rainfall during the growth period. The long periods of rainless weather in India are apt to distract attention from the necessity of drainage. In reality, however, in a country where most of the rainfall is compressed into three months, the necessity of perfect drainage is even greater than in localities where the total precipitation is more evenly distributed through the year.

At Pusa, during the wheat growing season, 1909-10, which was preceded by a heavy monsoon, alternate strips of wheat and gram (*Cicer arietinum*, L.) were sown on a plot of heavy wheat land which was imperfectly drained during the monsoon. It was observed that while the gram was exceedingly good the wheat was poor and stunted with yellowish foliage and exceedingly small ears. The total crop was only a small fraction of that obtained on the rest of the field where the surface drainage was sufficient. The markedly different behaviour of a cereal and a legume growing under the same conditions in the presence of sufficient soil moisture suggested that the explanation of the difference would be found in the nitrogen supply in the soil. Accordingly the matter was made the subject of an experiment in the following year, 1910-11.

The monsoon of 1910, although well distributed, was small in amount and no waterlogging took place as the showers were absorbed and practically no water drained off the surface. In consequence, the land had to be artificially waterlogged and this was done during the month of September by pumping water from the river on to the area under experiment. The land selected for the experiment was well ploughed in the hot-weather of 1910 and fallowed till the end of August when the central portion was embanked and artificially kept wet during the whole of September. After drying sufficiently, the waterlogged portion was harrowed and ploughed up and managed in the ordinary way till sowing

time. Across the middle of the plots a strip was manured with nitrate of soda just before sowing, the total amount added being four cwt. to the acre. At first, the waterlogged area did best due to the abundant moisture but after tillering it rapidly fell behind the areas on either side. The nitrated strip in the waterlogged area soon became well marked but was hardly distinguished on the weathered plots on either side. The yields obtained are given in the following plan:—

The result of waterlogging wheat land at Pusa in 1910.

Normal cultivation.

Waterlogged during September.

Normal cultivation.

34.45	15.55	29.14
SHADED AREA TREATED WITH 4 CWT NITRATE OF SODA PER ACRE		
35.82	25.17	26.53
34.45	15.55	29.14

The numbers in the plan are bushels per acre.

It will be seen that the effect of waterlogging for a month was to reduce the yield by about sixteen bushels to the acre while the nitrate of soda on this area increased the yield by nearly ten bushels. The effect of the manure on the non-waterlogged plots, as was expected, was very little. The results prove that the effect of waterlogging wheat lands in the previous monsoon is to interfere with the nitrogen supply of the crop and to lower the yield.

This result is of some general interest in Indian agriculture and particularly in those tracts of the plains like Bihar where rice and wheat are both grown. The lowlying areas in these tracts,

which receive drainage water from the higher lands, are generally planted in rice and these lands are often inundated and always waterlogged for long periods during the growth of the rice crop. The rice plant however thrives under these conditions and is able to take up its supply of nitrogen under waterlogged conditions, most likely in forms such as ammonia which are not suitable for other crops. In wet years like 1909 in Bihar the waterlogged and marshy conditions, associated with rice culture, may be said to have spread beyond and above the paddy fields and to have affected the wheat lands. This naturally influenced the soil processes and consequently the supply of available nitrogen for the wheat crop. Gram, however, being able to supply itself with nitrogenous food-material, was not affected and could thrive where a cereal like wheat to all intents and purposes starved.

From the economic standpoint the results of this experiment point to the great importance of drainage in the alluvial soils of India and the need of the limitation as it were of rice conditions to the areas which produce this crop. Where canals are used for watering the wheat crop it is also essential that the fields should be level so that all parts are equally watered. Where low areas exist, the surplus irrigation water drains into and waterlogs these areas and the result is a small crop of poor quality. On the black cotton soils of the Central Provinces it is often observed that the lowlying areas of the wheat fields often yield a larger proportion of spotted and soft grains than those parts which lie higher or are better drained. This partial waterlogging, which is more frequent in the black cotton soils than in the alluvium, is probably one of the chief causes of the unevenness in the consistency of the wheat often grown in Central India. The greater unevenness of the fields in Peninsular India probably follows from the fact that the levelling beam (*sohaga*) does not seem to be in general use in these regions. It is most important from the point of view of the miller that samples should be uniform in consistency otherwise a lower price is obtained for the wheat. The cultivator

in growing wheat of mixed consistency loses twice over. In the first place the yield is reduced and in the second place the quality is affected.

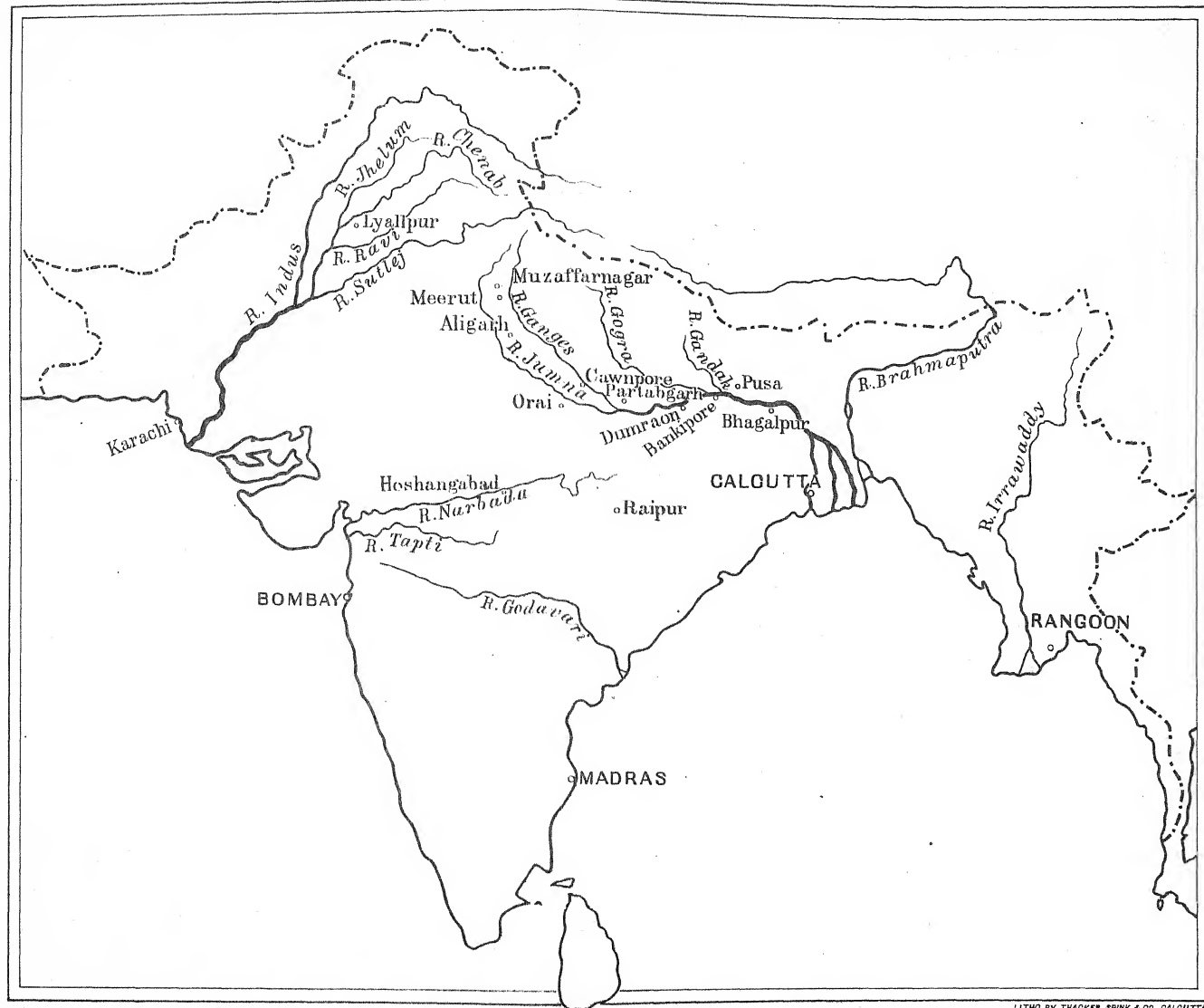
These experiments clearly indicate two of the main factors in the plains on which optimum yield and quality in wheat depend.¹ If cultivation is inadequate the yield falls and the quality is also affected. Want of drainage lowers the yield, affects the consistency and also lowers the quality. As regards wheat production it may be said that the best quality is obtained when the optimum yield is produced and that in any particular wheat, the ryot who produces the greatest yield has also secured the best quality possible in that wheat. If under these circumstances he grows a wheat in which high yielding power and high quality are combined he is then getting the greatest financial return for his labour.

¹Another important factor in wheat growing in the alluvium, in addition to hot-weather cultivation and drainage, may be mentioned. This is the treatment of the subsoil after it has been compacted by the monsoon rainfall. Experiments conducted at Pusa on the last three wheat crops, 1909-10 to 1911-12, have clearly indicated the advantage of a deep-ploughing towards the end of the monsoon. This aerates the subsoil, increases the root-range of the wheat plant and results in a considerable improvement in the standing-power of the crop as well as a better filled and more attractive sample. In the crop of 1911-12 the results of late deep-ploughing were particularly well marked. This cultivation must however be carried out without an undue loss of moisture—a matter of some difficulty in certain years with soil-inverting iron ploughs. On large estates it is possible that the best results will be obtained by the use of some form of sub-soil plough.

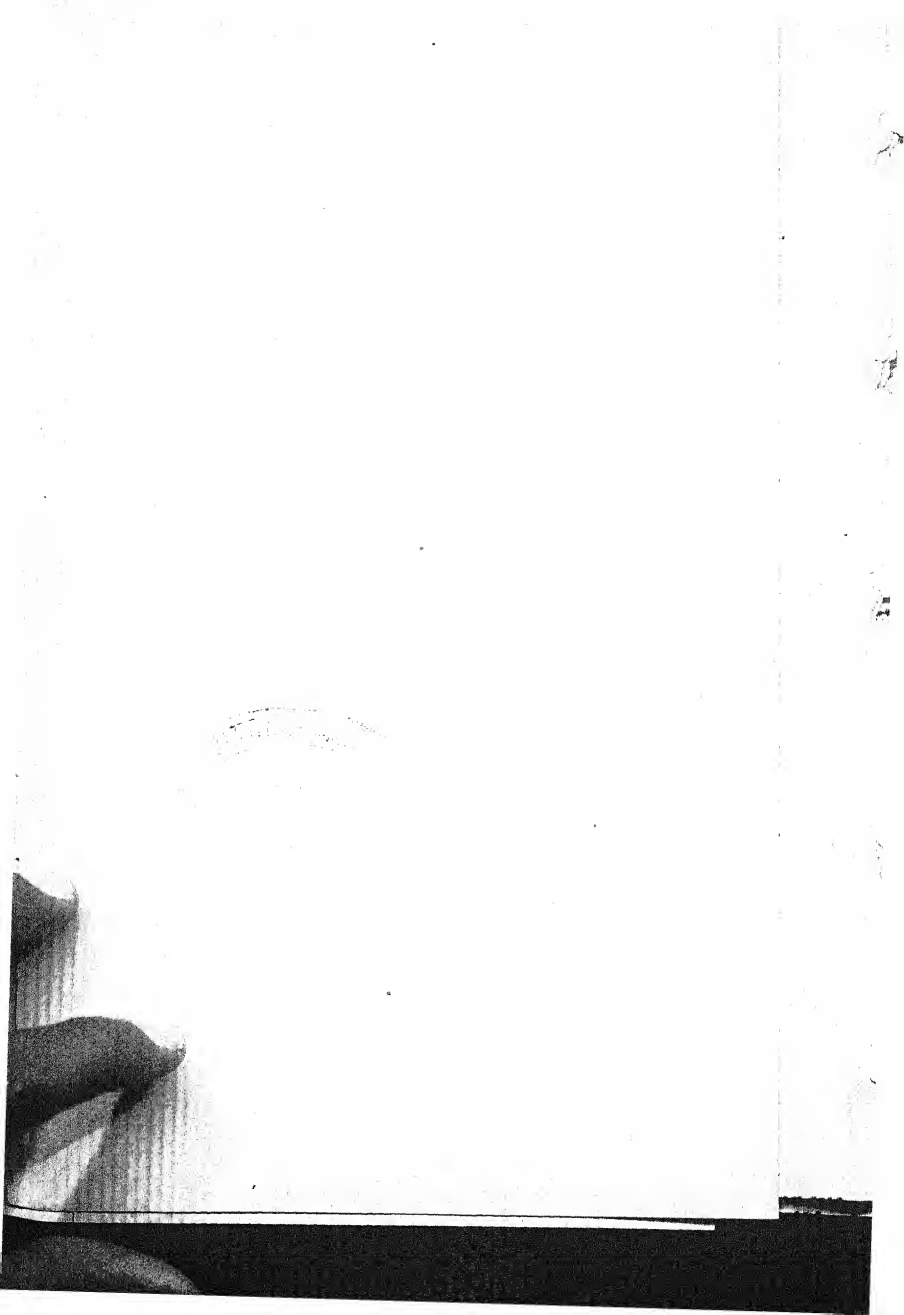
V. SUMMARY OF CONCLUSIONS.

The conclusions arrived at as a result of the investigations described in this paper may be summed up as follows :—

1. Usually in India the consistency of a wheat varies greatly according to the conditions under which it is grown. Some translucent wheats however are affected to a much less extent than others while a few soft wheats have always remained soft.
2. Weak wheats like Muzaffarnagar can be improved to some extent in milling and baking qualities by cultivation, but they have not been made to behave like strong wheats.
3. Strong wheats with good milling qualities have been found to retain strength and milling qualities both under canal irrigation on the alluvium and also on the black soils of Peninsular India. In the future improvement of the wheats of these tracts the question of grain quality should receive particular attention.
4. Adverse factors, such as waterlogging and late cultivation, affect both the yield and quality of wheat in the plains of India. In any particular wheat, the conditions which produce the highest yield are those which also produce the best sample. In the same wheat high yield and high quality can be combined. To obtain the greatest financial return for his labour the cultivator should grow to perfection a wheat which combines high yielding power with high quality.



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THE VARIETIES OF SOY BEANS FOUND IN BEN-
GAL, BIHAR AND ORISSA AND THEIR
COMMERCIAL POSSIBILITIES.

BY

E. J. WOODHOUSE, M.A., F.L.S

Economic Botanist to the Government of Bihar and Orissa

AND

C. SOMERS TAYLOR, B.A

Agricultural Chemist to the Government of Bihar and Orissa



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THE VARIETIES OF SOY BEANS FOUND IN BENGAL, BIHAR AND ORISSA, AND THEIR COMMERCIAL POSSIBILITIES.*

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E. J. WOODHOUSE, M.A., F.L.S.,

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AND

C. SOMERS TAYLOR, B.A.,

Agricultural Chemist to the Government of Bihar and Orissa.

1.—INTRODUCTORY.

IN 1909 the survey work on the crops of Bengal was commenced, and in July of that year a number of trial plots of the chief pulse crops of the Province were sown for identification and field study. Among these were three samples of *Bhetmas*, of which one sample of black seeded *Bhetmas*, received from the Settlement Officer, Bhagalpur, was found to be pure for seed characters and was sown as P. R. 135 ; the other sample which had been collected locally was found to contain chocolate and yellow seeds, and the two types were sown separately as P. R. 94 and P. R. 95. All the plots did well and were identified as varieties of the Soy Bean, but in October it became apparent that the black seeded type differed from the other two in having

* This paper was prepared for the Press in March 1912, but owing to the absence of one of the authors in England and to other causes its publication has been delayed.

darker bullate leaves and a more prostrate habit. At harvest time a limited number of single plants were selected with a view to further Botanical study. In the spring of the following year the interest taken in the Soy Bean crop elsewhere encouraged us to examine the chemical composition of these three types, and it was found that there was a marked difference in the proteid content of the types, the variation in the nitrogen content of the samples of each type being less than the differences between the means of the types.

In 1910, the seed of the single plants which had been analysed was sown together with some samples obtained by Mr. A. C. Ghosh from the Kurseong bazar and some more samples of the types already obtained from the plains. The seed of the original plots was also resown for further study. Acclimatised seed of two American varieties, Barchet and Riceland, were obtained from Saharanpur and sown for comparison with the local varieties. As a result of the year's work a distinctly earlier black seeded form was obtained from a plot (E 256 of 1910) grown from seed collected at the Bankipur Exhibition. The trial plots of the seed from Kurseong failed. More attention was paid to the chemical selection work and 150 single plants of each of the three types grown in the previous year were analysed for nitrogen and oil. The seed of each single plant derived from the seed of the original single plants selected in the previous year was again analysed. These analyses confirmed our conclusions of the previous year and showed us that the percentage oil content was also an inherited character.

In 1911, the seed of a number of the single plants analysed in the previous year was sown, and the seed of all the plants of each of the original selections was bulked and that derived from each of the original selected plants was again sown separately. These plots have again been analysed and confirm our original conclusions. In addition, the success of the plots of the acclimatized American varieties induced us to exchange seeds of the Bengal types with Mr. Piper, of the United States Plant Industry Bureau, who kindly supplied us with seed of the

varieties, Barchet, Duggar, Haberlandt, Hollybrook, Mammoth, Pekin, Pingshu. Another attempt was made to cultivate at Sabour the varieties grown in the Sikkim Himalayas, and plots of the Nepali, Barmeli, black seed, greenish yellow seed and chocolate seed varieties were obtained from Mr. Goodwin, Superintendent of the Kalimpong Homes Farm, and grown successfully at Sabour. Arrangements were also made with the Deputy Director of Agriculture for testing the yield of the Bengal types on the Department's farms. As a result of the work of these three years we have come to the conclusions, which are detailed in the following sections.

As regards the method of cultivation adopted, the land is levelled and prepared for sowing during the hot weather, and the seed sown as soon as convenient after the break of the monsoon. When the land is ready the drills are prepared four feet apart by means of the Planet Junior Hoe with plough share attachment. The seed is then sown by hand and the drills covered in with rakes. After cultivation consists in occasional hoeings with a Planet Junior Hoe (with plough share attachment), which slightly ridges up the plants and prevents water-logging. The plants are eventually thinned out to one foot apart in the rows. The rows have always been eighty-six yards long, and some four or five plots are usually sown in each row.

This work was carried out in the official years 1909-10 to 1911-12, previous to the repartition of Bengal in April 1912.

2.—NOMENCLATURE.

It is not the intention of this paper to discuss in detail the nomenclature of the Soy Bean, as that would not be possible without an exhaustive examination of the type specimens in European collections of Herbarium material. It will be sufficient to refer to some points of interest in the more important published descriptions of Indian Soy Beans which are available here. DeCandolle (1) describes *Soya hispida* Moench as an erect hispid herb. Roxburgh's (2) description of *Dolichos soja* Willd is taken

from plants grown at Calcutta from seed imported from the Moluccas, and these plants were of the twining hairy type. Wright and Walker Arnott (3) give the characters of the genus *Soja* Moench as annual, erect, flexuose, very hairy, and they mention only one species *S. hispida* Moench. In Bentham's (4) account of the cultivated *Glycine Soja* (Sieb. and Zucc.) the plant is described as villous. From Hooker's (5) description of *Glycine Soja* (Sieb. and Zucc.), it would appear that he had examined only the stout suberect type with membranaceous leaves, represented in the Sikkin Himalayas by the Nepali variety. Duthie and Fuller (6) give an account of the cultivated plant under the name *Glycine hispida* Moench, and describe it as an annual, covered with ferruginous hairs, with stems stout, suberect, and climbing. Their plate represents one of the hairy twining forms found in the plains of India. Church (7) describes *Glycine soja* (Sieb. and Zucc.) as a small suberect, trifoliate, hairy, annual and gives a plate which probably represents one of the twining hairy forms commonly found in the plains. Prain (8) mentions *Glycine hispida* Maxim as a suberect annual cultivated occasionally in Western Bengal, and in another place (8a) states that a specimen of *Glycine* collected on the banks of the Ganges at Sahibganj has long trailing stems, and "but for their hispidity might pass as representing the wild *G. ussuriensis*."

Piper and Morse (9) come to the conclusion that the cultivated Soy Bean is *Glycine hispida* (Moench) Maximowicz, and that its nearest relative is *Glycine soja* Siebold and Zuccarini (*G. ussuriensis* Regel and Maack). They distinguish these two species by saying that *G. soja* has more slender and more vining stems, is less hairy, bears smaller pods and seeds and has smaller flowers, the calyx lobes being shorter in proportion to the tube than in *G. hispida*. They conclude that the best critical character is the length of the flower, which is 3 to 5 mm. in *G. soja* and 6 to 7 mm. in *G. hispida*, but that if this character is used *G. soja* is also a cultivated species. They consider that there is no good reason why *G. hispida* may not have been derived from *G. soja* by cultivation, the smaller flowers of the latter being the principal

difficulty. In this connection it may be noted that no differences in the lengths of the flowers of the many cultivated varieties have yet been observed at Sabour. In view of this fact and the very numerous and marked differences there are in habit, hairiness and seed characters, which will be discussed in section 3 of this paper, it would seem advisable to accept the final conclusion come to by Messrs. Piper and Morse that "on the whole, we are therefore inclined to believe that there is but one botanical species, which has been profoundly modified by cultivation."

3.—VARIETAL CHARACTERS OF SOY BEANS.

3a.—HABIT.

In germination the fleshy oblong cotyledons are carried up above ground by the stout hypocotyl, which is usually of a purple colour. The plumule and upmost portion of the hypocotyl is hairy. The American varieties Mammoth and Hollybrook can be distinguished by their stouter cotyledons and hypocotyl. The first pair of true leaves are simple and cordate-cuneate in shape, the subsequent leaves being alternate and trifoliate. In all the varieties introduced into cultivation at Sabour and on the other farms in Bengal, nodules were found to be present on the roots.

The work on Soy Beans has not progressed sufficiently far to enable us to be certain of the factors which produce the many differences of habit, but it would appear that there may be two principal pairs of characters, upright and reclining stems and branches, short and long internodes. As regards the first pair of characters, two of the varieties grown in the Sikkin Himalayas, Barmeli (type 5)* and Nepali (type 6) and all of the American varieties have upright stems, while the four types (I—IV) usually found in the plains of India have reclining branches, but of these the chocolate and yellow seeded types (III, IV) have the lower part of the main stem slightly more upright than

* Type 5 refers to the descriptive list of the types of Soy Beans cultivated in Bengal (vide Section 4).

in the case of the black types (I, II). In the case of the second pair of characters, the Barmeli and Nepali varieties and the American varieties Barchet, Hollybrook, Mammoth and Riceland combine short internodes (usually less than 1 inch) with the upright habit, although most of the American varieties are not pure for these characters. The Bengal types I—IV combine greater length of their upper internodes with the reclining habit. In the case of some of the American varieties (and rogues found in most of them) and also in the greeny yellow seeded variety (type III A) from Kalimpong, the upright habit is combined with long twining branches. In the rogues commonly found in the Barmeli variety the reclining habit is combined with short internodes. That there are other characters which determine the number and length of the branches relative to the main stem can be assumed from a study of plate IV of Piper and Morse's bulletin (9). A continuation of the study of the branching of the different types will undoubtedly bring to light additional characters. The importance of those mentioned above depends on the fact that the short stemmed plants mature earlier. The upright habit enables the plots of this type to be weeded more conveniently. The long much branched twining forms produce a larger number of pods per plant, and may be especially suitable for growing with maize, when they will yield well and will also not interfere with the weeding of the crop. These long branched forms are also more suitable for fodder purposes.

3b.—FOLIAGE.

The chief differences found in the foliage are in the shape, size, colour, surface and degree of persistence of the trifoliate leaves. The shape of the apical leaflets may usually be described as ovate lanceolate. In the Bengal types (I—VI) the shape is approximately the same in all cases, though the later leaves of types III, IV are often narrower than the earlier leaves. In the case of the impure yellow (type III A) and chocolate seeded (type IV A) varieties from Kalimpong some narrow leaved plants have been selected. In the American varieties narrow

leaved plants are very common and one plant (E 468 of II) selected in 1910 bred true to this character in the year 1911. In size the leaves of the Barmeli and the Nepali varieties are larger than the other Bengal types. The colour of the leaves of the black seeded types (I, II) is slightly darker than that of the chocolate and yellow types (III, IV). The distinctive colour of the Nepali and American varieties is due to absence of pubescence. The black seeded types (I, II) have bullate leaves, which have hitherto distinguished them from other types, but in a natural cross (E 426 of II), this character has been found in other combinations. The upright types have upright petioles from which the leaflets are deciduous.

3c.—PUBESCENCE.

The differences in the hairiness of Soy Bean varieties may be classed under two heads, the colour and the quantity of the pubescence. As regards its colour Piper and Morse (9) have shown that tawny pubescence is a Mendelian dominant to white pubescence. With the exception of Barmeli (type V), in which the hairs become whitish as the plant ripens, all the Bengal types have tawny pubescence. Differences in the quantity of the pubescence do not appear to have been noticed in America, but in the Bengal types I—V the leaves differ from the American varieties and Nepali (type VI) in being covered with soft upright hairs on their upper surfaces, whereas the upper surfaces of the leaves of the latter types are covered with closely adpressed hairs. The American varieties and Nepali can be distinguished at a distance from the fully pubescent types by the darker green colour of their leaves.

3d.—FLOWER.

1. *Morphology*.—The flowers are produced on short axillary or terminal racemes which in Barmeli are sometimes longer than in the other types.

The flowers of all the varieties grown at Sabour measured from 6 to 7 mm. The flower colour of the Bengal types I—V was purple, though the Barmeli type is slightly redder. The Nepali

in the case of the black types (I, II). In the case of the second pair of characters, the Barmeli and Nepali varieties and the American varieties Barchet, Hollybrook, Mammoth and Riceland combine short internodes (usually less than 1 inch) with the upright habit, although most of the American varieties are not pure for these characters. The Bengal types I—IV combine greater length of their upper internodes with the reclining habit. In the case of some of the American varieties (and rogues found in most of them) and also in the greeny yellow seeded variety (type III A) from Kalimpong, the upright habit is combined with long twining branches. In the rogues commonly found in the Barmeli variety the reclining habit is combined with short internodes. That there are other characters which determine the number and length of the branches relative to the main stem can be assumed from a study of plate IV of Piper and Morse's bulletin (9). A continuation of the study of the branching of the different types will undoubtedly bring to light additional characters. The importance of those mentioned above depends on the fact that the short stemmed plants mature earlier. The upright habit enables the plots of this type to be weeded more conveniently. The long much branched twining forms produce a larger number of pods per plant, and may be especially suitable for growing with maize, when they will yield well and will also not interfere with the weeding of the crop. These long branched forms are also more suitable for fodder purposes.

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3d.—FLOWER.

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The flowers of all the varieties grown at Sabour measured from 6 to 7 mm. The flower colour of the Bengal types I—V was purple, though the Barmeli type is slightly redder. The Nepali

type has white flowers and this colour is common among the American varieties, which are frequently impure for this character. In young buds, of which the corolla is just showing colour, the pistil projects considerably beyond the stamens as they just begin to dehisce; but the stamens gradually elongate and in the flowers the stamens will be found to be very nearly as long as the pistil, and the stamens and pistil will be found to be completely covered with yellow pollen grains. In some cases the pollen is pushed out of the end of the keel by the elongation of the stamens. The standard forms a roof which lies close over the top of the keel.

2. *Pollination*.—Piper and Morse found the Soy Bean flower completely self-fertile, bagged plants setting pods as perfectly as those in the open; they also found that the flowers are much visited by bees for the purpose of eating the pollen, but cross-pollination is prevented by the abundance of the pollen which covers the stigma almost as soon as the flowers open. The observations made at Sabour in 1910 showed that the seed would set perfectly well under bag, but the results of bagging were not so satisfactory in 1911, probably due to the heavier rainfall. From the above description of the flower it also appears that the stamens open early and their elongation brings large quantities of pollen in contact with the stigma; it is therefore probable that the seeds are normally self-fertilized unless visited by insects before the pollen has come into contact with the stigma.

Observations were made by Mr. H. L. Dutt on the insects visiting the flowers, and it was found that the number of insects visiting the flowers was largest between 10 and 12 A.M. Among bees the following species were noticed:—*Apis dorsata*, F.; *Apis indica*, F.; and *Nomia cognata*, Bing. Of these, *Apis dorsata* was not common. It clung to the keel with its first two pairs of legs, with its abdomen hanging down below the keel, while it inserted its head between the standard and keel. *Apis indica* was more commonly found than the above species. *Nomia cognata* was the most common species visiting the flowers, as

many as six insects being found together on one plant in the mornings. Lepidoptera were represented by a few specimens of *Parnara mathias*, F. ; *Pachyzancla stultalis*, Wik, and *Zinckenia fascialis*, Cram. A chrysomelid beetle and *Epilachna dodecostigma*, Muls., were common on the leaves of the plants. Several muscid flies were almost as plentiful as the bees to which their behaviour was similar. It is probable that bees and flies sometimes cross-fertilize some of the younger flowers.

3. *Extent of Natural Crossing*.—Messrs. Piper and Morse's observations at the Arlington Experiment Station have made them conclude that even when the test rows are grown contiguously, the percentage of hybrids is perhaps not one individual in two hundred. They also find that the hybrid seed can be recognised by the presence of peculiar markings on the seed. Heterozygote plants can be distinguished by the appearance of the pods at the top of the branches, which pods are more tumid, less hairy and of thinner texture. Our observations at Sabour lead us to conclude that natural crosses do not occur on the plains of India to such an extent as in America. The three varieties which have been grown next to next since 1909 were found to be pure for all characters in that year and have since retained those characters. In 1910 eleven single plant cultures were grown of the three types I, III & IV, but no rogues were found among them. In 1911 sixty-four plots derived from single plants in 1909 or 1910 were grown in lines next to next and of these only one plot was found to be a hybrid. This plot (E 426 of II) was grown from the seed of a single plant of the yellow variety (type III), which was selected on account of its greener colour and darker hilum. Isolated rogues were also found in a few other plots and will be tested in 1912. It will probably be found that most of them are chance admixtures. A statement of the rogues found in 1911 is given below. In all, 3,587 plants were harvested from unbagged seed grown from single plants in 1910. Column 3 gives the number of years during which each single plant culture has been grown.

1 Field number.	2 Description.	3 Period of Exprt.	4 Composition of plot.
E414 of 1911 ...	Type III (yellow) ...	2nd year .	180 plants, seed yellow, hilum pale brown. 1 seed yellow, hilum browner.
420 of " ...	Ditto ...	1st " ...	4 " " " pale brown. 1 " " " browner.
430 of " ...	Type IV, chocolate ...	2nd " ...	181 " chocolate. 3 " black.
432 of " ...	Ditto ...	2nd " ...	160 " chocolate. 1 " greeny yellow. 1 " black.
438 of " ...	Ditto ...	1st " ...	88 " chocolate. 1 " yellow.
426 of " ...	Greeny yellow, hilum dark brown.	1st " ...	7 " black. 2 " chocolate, hilum chocolate. 9 " greeny yellow clouded black hilum black. 3 " greeny yellow clouded chocolate, hilum chocolate.
466 of " ...	Barchet ...	1st " ...	6 seed dull olive brown (Barchet). 1 " " black (Riceland).

It is interesting to note that the American varieties and those obtained from Darjeeling district were full of impurities, whereas all the samples of seed (pure for seed characters) collected in the plains have been found to be pure for vegetative characters also. It may be that crossing occurs more frequently in the more temperate climate of America and the Darjeeling Himalayas.

3c.—Pods.

No marked differences have been noticed in the shape or size of the pods of the varieties cultivated at Sabour. The differences in the colour of the pubescence have been noted above. The colour of the pods differs from the usual yellow colour in some cases. The Barmeli type resembles the American varieties Mammoth and Hollybrook in having whitish pods, and the Riceland variety differs from Barchet in having darker brown pods. The pods appear to shatter less easily in the twining types (I—IV) than in the upright varieties. From one to three pods are usually produced by each inflorescence, though this number is often increased to seven in the case of the Barmeli type. The pods never contain more than from one to three seeds in the types examined.

The production of pods per plant differs markedly in different types depending on the extent of branching of the plant. In the upright types with few short branches, the maximum number of pods produced in a well developed plant very seldom exceeds five hundred and in good average specimens of the Barchet, Riceland and Barmeli varieties, it is probable about two hundred and fifty, yielding about 40 grams of seed. In the Nepali type grown at Sabour about fifty pods were produced on each plant. The longer and more profusely branched types (I—IV) can produce up to a maximum of one thousand five hundred pods, yielding up to 175 grams of seed per plant. The yellow and chocolate types (III & IV) also have given the best yields in the Botanical test plots producing about 50 grams of seed per plant, while the black types produced only about 30 grams per plant. The actual figures obtained in field trials (about 25 acre plots) of the three types are given in the statement below :—

Plot.	Plants counted.	PODS PER PLANT.		Weight of seed per plant average of 100 plants.	Yield per acre.	REMARKS.
		Extremes.	Mean.			
Type 4 chocolate.	12	135—1312	557	32 grams	13 mds. 16 srs.	1 md. = 82½ lbs. = 40 srs.
Type 3 yellow	12	71—911	392	23.5 "	10 " 39 "	...
Type 1 black	12	90—670	200	13 "	5 " 39 "	Germination poor.

The figures for the number of pods per plant found in the Bengal types may be compared with the average yield of pods per plant given by Shaw (10) from the Tichling district of China—

1908	...	35—	80 pods per plant.
1909	...	40—76	" " "
1910	...	42—105	" " "

The results of our observations on pod production would appear to indicate that it will be desirable to raise types

combining the upright habit with the capabilities of producing the higher orders of branching possessed by the Bengal twining types (I—IV). It should be noted that the production of pods in the best of the types at present under cultivation compares favourably with that of the field peas of which the weight of seed per plant has not been found to exceed 150 grams per plant, and is distinctly superior to that of other Indian Kharif pulses except Rahar (*Cajanus indicus*).

3f.—SEEDS (MORPHOLOGY).

In shape the seeds are usually elliptic in outline, the thickness being less than the breadth; but the seeds vary in thickness from the much flattened seeds of the black twining varieties (types I, II) to the Nepali variety which is round in section.

The size and weight of the seed vary considerably, the lowest weight per hundred seeds being less than 4 grams in the case of the twining types I—IV, and as much as 24 grams in the case of Nepali. Hooper (11) observed that black seeds collected from the hills are heavier than those collected in the plains. Our observations also show that the black seeded variety (type I A) obtained from the hills which appears to be practically the same type as that cultivated in the plains (type I) has a weight per 100 seeds of 6.25 grams, but after one season's growth it has been reduced to 5.5 grams per 100 seeds, as compared to an average of 3.9 for the plots of the local variety. The greenish yellow (type 3 A) and pale chocolate (type 4 A) varieties introduced from the hills have behaved similarly, but these differ from the yellow and chocolate varieties of the plains in vegetative characters. The early black twining type (type I) obtained from Patna has increased in weight from 3.2 grams per 100 to 3.9 grams under cultivation at Sabour. The weight of 100 seeds in some of the American varieties such as Barchet, Pekin and Pingshu has remained approximately constant, whereas others, such as Duggar, Hollybrook and Mammoth have decreased in weight more or less considerably during the past season. The Nepali type has decreased in weight from 24.4 grams to 12.8

grams per 100 seeds. The variation in weight per 100 seeds in the case of varieties cultivated in new localities would appear to give a good indication of the adaptability of a variety to its new environment.

There are many characters for seed colour, the local types being either black, chocolate or yellow. Yellow seeds may have more or less of a greenish tinge. In one Darjeeling variety the seeds are greenish yellow. In the case of the chocolate and yellow seeds there may be subtypes in which the colour is paler. A dark coloured seed coat may be a disadvantage if a white meal is required. The hilum may be of the same colour as the rest of the seed coat or the black or chocolate colour of the hilum may merge into the yellow colour of the rest of the seed coat. In these cases the hilum colour usually spreads out to form an uneven band round the hilum of greater or less width. The colour of the germ was yellow in all the varieties examined.

3f.—SEEDS (COMPOSITION).

Owing to the fact that the Soy Bean has recently come into prominent notice from its value as a food-stuff and also as an oil-yielding crop, it will be unnecessary to dilate upon these uses, as many able writers have called our attention to the use of Soy Beans for both purposes. In recent years Hooper (11) has published numerous analyses of Soy Beans from various parts of the world, a large pamphlet has been published upon the crop by the Imperial Chinese Customs (10) and numerous Bulletins, principally dealing with the purely botanical or agricultural side of the question, have been issued by the United States Department of Agriculture.

All chemical work published up to the present, however, appears to have been done on the broad lines of analyses of bulk samples introduced from different parts of the world and very little care appears to have been taken to ensure the purity of the types analysed. It was thought that it might prove of interest to examine the chemical characteristics of certain varieties grown from the seed of single plants.

In consequence several observations were made upon small quantities of seed obtained from single plants in 1909. Owing to the fact that a proportion of the seed was required for sowing again in the following year, the sizes of the samples were very small and we were only able to determine the nitrogen in small quantities of seed from each plant. From five to seven seeds were taken in each case, and the nitrogen was determined by Kjeldahl's method. It will be seen that it was impossible to arrive at definite conclusions from observations of such extremely small samples, which did not exceed 0.4 gram in weight in any case. Owing, however, to the fact that they were the starting point of our work, it will be of interest to give the results obtained from these experiments.

These results were as follows :—

Colour of Beans.	Original No.	Nitrogen content.	Variation from Mean.
Black (type 1)	135 A.	6.08	+.06
	135 B.	5.92	-.02
	135 C.	6.43	+.29
	Mean ...	6.14	
Yellow (type 3)	95 A.	5.99	+.15
	95 B.	5.93	+.09
	95 C.	5.60	-.24
	Mean ...	5.84	
Chocolate (type 4)	91 A.	5.32	+.20
	91 B.	5.41	+.32
	91 C.	4.83	-.20
	91 D.	4.74	-.38
	91 E.	5.28	+.16
	Mean ...	5.12	

The great difference in nitrogen content between the black and chocolate varieties led us to think that it would be of interest to see if these differences were merely accidental or were inherited characters of the varieties.

Owing to the small size of the samples, to which reference has already been made, it was thought that it would be of interest to corroborate our observations by an examination of

large bulk samples from the plots from which these single plants had been selected.

The results obtained were as follows :—

COLOUR.					Nitrogen. Per cent.
Black	6.17
Yellow	5.44
Chocolate	5.74

Although these plots were not grown from single plants, yet they appeared to be perfectly even, and these results, in which the black seed stood out ahead of the other two as regards its nitrogen content, tended to corroborate our observations on the small samples obtained from the single plants. Consequently in the rains of 1910 we resowed not only the seeds of the singly selected plants, but also seeds from the original plots from which they were chosen.

The descendants of the former were analysed plant by plant and the means are shown in the tables (Tables I—IV), giving their history for the past three years. From the descendants of the latter, 150 plants were taken from each plot for analysis (Tables V—VII).

The results of these analyses are striking, and are of additional interest in that we were able this time to determine the oil contents of a great many specimens. In order that reference may be made to these tables, they are published at the end of the letter-press.

On collecting the results we were still further strengthened in our belief that it was possible that certain differences in oil and nitrogen content might be correlated with certain definite vegetative characters.

Firstly, in considering the nitrogen contents of three varieties, we can arrange them in the following tabular statement showing the groups into which the samples of each variety may be arranged according to their nitrogen content (*see page 118*).



Tabular Statement showing grouping of samples of Soy Beans according to their nitrogen content.

[illegible]

From this we see that in 1910 the black seeded variety showed a tendency to group round the high nitrogen values while the yellow and chocolate varieties, although nearly equal to one another, were far below the black.

The actual means were as follows :—

Variety.	Nitrogen content.	Probable error of mean.
	Per cent.	
Black (Type 1) ...	6.72	+ .02
Yellow (Type 3) ...	5.61	+ .02
Chocolate (Type 4) ...	5.57	+ .02

The fluctuations from the mean were extremely small. The standard deviation was about $\pm .3$ and the probable error of a single sample was therefore about $\pm .2$.

Actual examination of the results obtained showed the following :—

Variety.	Mean $\pm .2$.	Outside mean $\pm .2$.
Black	64	76
Yellow	86	63
Chocolate	76	68

It has been mentioned that in 1911 we were able to determine the oil contents of each type of seed. For this purpose about 70 plants of each kind were taken at random and their oil content was determined. In this case it was found that the yellow and chocolate coloured varieties were by far the greatest in oil content, while the black seeds were in nearly every case far below them.

The means obtained were as follows :—

Variety.	No. of samples.	Mean oil content.	Probable error of mean.
		Per cent.	
Black	77	13.52	+ .08
Yellow	65	16.90	+ .07
Chocolate	68	17.13	+ .09

Now from the data at our disposal we were able to draw certain frequency curves for each variety.* These curves illustrate very clearly the fact that while the black seeds tended to have higher nitrogen values than the others, exactly the contrary was the case with regard to the oil values. Reference will be made to this later.

It has already been mentioned that in addition to these experiments undertaken in 1910 the actual descendants of the single plants originally analysed were sampled and their nitrogen content determined immediately.

Owing to lack of time at our disposal we were not able to determine the oil content of these plots, but observations on their nitrogen content led us to believe that they were not in any way different from the groups in which a determination of both nitrogen and oil was made.

It would appear, however, that in 1910 the nitrogen content of the black soy beans was abnormally high. This was not only the case in one plot, but was found in every single plot analysed. Such variations are only to be expected as the character of the season varies from year to year.

From the experiments made during the three years over which the crop has been under observation, however, it would appear extremely probable that the tendency of the black seeded variety is to maintain a high percentage of nitrogen and low percentage of oil, while the other two varieties probably have a tendency in a similar degree to give lower percentage of the former and higher of the latter constituent. There appears to be in fact little difference between the so-called chocolate and yellow varieties as will be seen from the table already shown. This is of great interest when viewed in conjunction with the fact that the vegetative characters of the yellow and chocolate varieties, apart from the seed colour, appear to be similar, and distinctly different from those of the black. From this we are led to believe that such characteristics as the oil and nitrogen content of the crop may be correlated with actual vegetative characters.

* These curves are shown at the end of the Memoir.

That each variety contains unit species which differ in their nitrogen and oil content from the mean of the variety is not only extremely unlikely but is negated by certain experiments made by us on certain seeds which were found to differ widely from the normal (*vide* Tables I, II and III).

It will be seen from what has gone before that the variety, having a high oil content had a low nitrogen content, and that the contrary was also the case. This led us to wonder whether the differences observed in 1910 and more carefully in 1911 might have been due to some external cause which increased the formation of nitrogen and at the same time decreased the formation of oil. If this were the case the correlation between nitrogen and oil content in any single variety should have a very high negative value. The correlation was in consequence worked out in each variety by the usual formula.

The results in each variety were very similar in magnitude. They were as follows :—

Variety.	Correlation Factor.	Probable Error.
Black (Type 1) ...	-.25	+.07
Yellow (Type 3) ...	-.31	+.07
Chocolate (Type 4) ...	-.33	+.07

There is therefore an indication of a slight negative correlation between the nitrogen and oil contents, but from these data they cannot be considered to be complementary to one another. In other words it is extremely improbable if, by some external cause, we were to be able to increase the average nitrogen content of the yellow soy bean crop until it reached the average observed by us in the black seeded variety, that the oil content would diminish at the same rate. Some slight correlation is naturally to be expected, as the increase in one form of energy supply is likely to be balanced by a decrease in the other form; but the above relations point clearly to the fact that the correlation is not by any means absolute. In addition to this another test was made by bringing into consideration the

size of the seeds whose oil content had been determined. If external conditions affect the plant at all, they are very likely to show their action upon the weight of the individual seed, or upon its total yield. As a case in point, we may instance a soy bean (Type VI Nepali) whose seeds when grown at Sabour are only half the weight of those grown in the hills.

From about 50 samples of the same type the correlation factor between the size of seed and oil content is found to be extremely small.

Hence it follows that although in the case of three definite types of soy beans the mean oil content was found in two cases to be relatively high and the nitrogen content was low, while in the remaining unit species the reverse phenomenon was observed, yet in any particular type there was not any great indication of the dependence of oil and nitrogen content upon one another or upon such external conditions as would modify the size of the seed. We are, therefore, led to believe that in the three types studied, the percentage content of nitrogen and oil is in all probability a characteristic that is inherited and that the variations which we have observed have been purely due to the differences in the types examined, and not to external causes.

Finally in 1911 the means of the bulked plots from the seed of single plants were found to be as follows :—

Type.	No. of plots.	Nitrogen content. %	Probable error of mean.	Mean oil content. %	Probable error of mean.
I Black ...	15	6.32	±.06	12.85	±.13
III Yellow ...	12	5.68	±.03	16.65	±.10
IV* Chocolate ...	33	5.95	±.03	16.20	±.10

* This includes 24 separate plots each grown from single plants grown from the original No. 94C. The results of each of these plots are given in Table IV.

From this it will be seen that the mean nitrogen content of the black type again far exceeds that of the others, while the differences as regards oil content are almost identical with those observed last year.

The actual divergencies from the mean were very small and were in most cases within the limits of experimental error. Taking for instance the case of the black soy beans, which mean nitrogen content was 6.3% (approximately), we find that out of 15 determinations 13 were within the limits $3 \pm .2\%$, and each of the fifteen came within the limits $3 \pm .3\%$. In the same way the mean of the results obtained with the yellow soy beans was 5.7% (approximately), and out of 12 plots sampled and analysed 10 were within the limits $7 \pm .2\%$ and the whole twelve came within the limits $7 \pm .3\%$. Again in the chocolate coloured variety we find that out of 33 plots sampled and analysed, whose mean nitrogen content was approximately 6%, 26 came within the limits $6 \pm .2\%$ and 30 within the limits $6 \pm .3\%$.

From this it follows that the variation in nitrogen content of individual plots was very small, and for all practical purposes may be considered to have been constant over the whole field.

Similar constancy is observed in the oil determination, and of necessity cannot be so striking, owing to the fact that the methods used in the determination of oil are by no means so accurate as those used for the estimation of nitrogen.

3g.—MATURITY.

The life period of the different varieties of Soy Beans varies over a wide range. Piper and Morse (9) state that the early American varieties ripen in eighty days and the latest varieties over one hundred and fifty days, while we have found that the varieties (Types I—IV) found in the plains of Bengal take as much as two hundred days to mature. These types do not reach maturity in America. These late types have a serious disadvantage in that they remain on the land during both the rainy and cold seasons so that only one crop can be taken on the land during the year, but, on the other hand, they would appear to produce more pods per plant than the earlier types. Before Soy Beans can be recommended for cultivation in India on a large scale

it will probably be necessary to introduce satisfactory early types.

Soy Beans are grown during the rainy season in Bihar. The local varieties (Types I—IV) resemble the other *Bhadai* pulse crops in that they ripen off at the same time of year irrespective of the date on which they are planted. These late varieties normally ripen after the middle of December, but can be sown from the end of May to the beginning of September. If they are planted late in September or early in October the size of the plant is much reduced and the date of harvesting may be somewhat later. The varieties grown in the Darjeeling hills have a somewhat shorter period of growth and were ripe in November at Sabour in 1911. None of these short season varieties have been found to have spread into the plains. A statement is given showing the life periods of the Bengal types. (Table VIII.)

As a result of some observations made by Ball (12) and Piper and Morse (9) in America it has been thought that a variety may progressively change from early to late, but the evidence is at present by no means conclusive. The observations made by us at Sabour show that newly imported American varieties take a considerably shorter time to mature at Sabour than in America, but that plants from acclimatized seed mature somewhat later than those from freshly imported seed. A statement of our observations is given below :—

Life periods of American varieties of Soy Beans grown at Sabour, 1911.

Variety.	Origin.	Date of planting.	Date of harvesting.	Life period at Sabour.	Life period in U. S. A.	REMARKS.
Barchet ...	S. P. I. No. 23232	July 12	Oct. 18 - 28	99-108 days.	Over 150 days.	
Do. ...	Acclimatized from Saharanpur.	"	Nov. 9 17	120-128	do.	
Duggar ...	S. P. I. No. 17268c	"	Oct. 11	91	110-120	
Haberlandt ...	S. P. I. No. 17271	"	Oct. 31	111	120-130	
Hollybrook ...	S. P. I. No. 17278	"	Oct. 19	99	130-150	
Mammoth ...	S. P. I. No. 30205	"	Oct. 25	105	130-150	
Pekin ...	S. P. I. No. 17852B	"	Sept. 30	80	...	
Pingshu ...	S. P. I. No. 18259	"	Sept. 25	75	...	
Rice land ...	Acclimatized seed from Saharanpur.	"	Nov. 13	124	Over 150	

3h.—DISEASES.

Soy beans do not appear to suffer much from diseases, though a portion of the chocolate-seeded plot was wilted by *Fusarium* sp. in 1909, and again in 1911 a portion of one of the chocolate-seeded plots was attacked. A leafspot disease caused by a species of *Cercospora* produces brown spots on the leaves and weakens the plants somewhat. No very serious damage to the plots at Sabour has been done by insect pests though surface grasshoppers (*Chrotogonus trachypterus*) do some damage to the young seedlings, and it is necessary to keep picking off the young broods of the Bihar hairy caterpillar (*Diacrisia obliqua*) as they appear. Mr. Dutt has also observed groundnut leaf roller (*Anarsia ephippias*) on soy beans at Pusa. One of the great merits of the soy beans examined at Sabour is the entire freedom of the stored grain from weevils, a fact which is of interest in view of Shaw's (10) statement that in China "worms and weevils do damage to the seeds after harvest."

4.—DESCRIPTION OF TYPES.

It has been thought advisable to describe the chief types of soy beans isolated up to the present time (season 1911) in order to record the characters of the types on which the chemical selection work has been carried out. In the course of a few years, when the various combinations of the very large number of unit characters have been isolated, it is probable that a very large number of types will be known.

TYPE I.—"*Kala Bhetmas*."

Stem and secondary branches long, weak, reclining, some tertiaries ascending. Leaves bullate. Stems, leaves and pods with tawny pubescence. Flowers purple 6 to 7 mm. long, usually sessile in short racemes. Pods 1·3" to 1·7" long, 1—3 together, seeds black, 4 to 6·5 mm. long by 3 to 4·5 mm. broad, elliptical, much flattened, germ yellow. Seeds contained 6·1 to 6·6% nitrogen and 11·2 to 13·5% oil in 1911.

The habit of plants of this type is quite distinct. The main stem and secondary branches lie along the ground, but some of

the shorter tertiary branches are more ascending and attain to a height of about 18 inches from the ground. A plot of this type is slightly taller than the early black but lower than the other types. Measurements were made of a typical plant at the end of September, and a main stem of 26 internodes was 33 inches long, the secondary branches were produced from the 2nd to the 17th node and measured up to 32 inches, the lower internodes of the main stem were less than 1 inch and the upper ones as much as 3.5 inches long. The reclining stems often root at the nodes. Red stains are often produced wherever the stems are injured. The lower leaves are usually darker green, and a typical measurement of an apical leaflet of a leaf subtending of a lower branch is 3.7 inches by 2.3 inches. The leaves at the apices of the branches are slightly smaller (3.3 inches by 1.6 inches), are distinctly bullate and are usually dotted with yellow. The whole plant is covered with tawny pubescence. The plants commence flowering at the end of September and are usually in full flower at the beginning of October; the terminal inflorescences are produced at the end of October and the pods ripen by the middle of December. The brownish pods are slightly curved and from one to three seeded. Not more than three pods are usually produced on one raceme. Unripe seeds are larger and purple in colour but become blacker and smaller as they ripen. The weight of seed produced per plant for an average of about 250 plants grown in the Botanical Experiment Area in 1911 worked out at 25 grams per plant. The weight of 100 seeds varies from 3.2 to 4.9 grams (mean 3.9). In connection with the fact that the seed has given a consistently high percentage of nitrogen, it is interesting to note that the prepared *dal* is considered by cultivators to be more heating than that of the other varieties. This type has been collected most frequently at exhibitions in the province. It has been grown at Sabour during the seasons 1909, 1910, 1911, and has kept its distinctive characters.

It appears to be grown as a pure crop without impurities, though types which vary in their time of ripening may occur. (Plate 1).

TYPE 1A. A batch of similar but slightly larger seed obtained from Darjeeling was found also to have bullate leaves, but to be made up of a number of constituents differing in time of ripening, the seeds were larger than the acclimatized type and weighed from 3.75 to 5.90 grams per 100. This variety is listed as variety 3 by Goodwin (13) in the Bengal Quarterly Journal, Vol. IV, No. 3.

TYPE 2.—“*Kala Bhetmas.*”

Stem and branches long, weak, reclining, leaves bullate. Stems, leaves and pods with tawny pubescence. Flowers purple, 6 to 7 mm. long, usually sessile in short racemes. Pods 1.2 to 1.5 inches, 1-3 together. Seeds black, 3.5 to 7 mm. long, by 3 to 4.5 mm. broad, elliptical, much flattened. Germ yellow. The seeds contained 6.1 to 6.5 per cent. of nitrogen and 12.4 to 14.3 per cent. oil in 1911.

This type differs from Type 1 only in having a more prostrate habit and in ripening considerably earlier. When this type was first selected the seed was found to be smaller than that of Type 1, but this difference has disappeared during its second year of cultivation. In 1910 seven individual plants of this type gave an average weight per 100 seeds of 3.2 grams (extremes 2.45 to 3.9 grams), whereas the offspring of these 7 plants in 1911 gave an average weight of 100 seeds of 3.9 grams (extremes 3.4 to 4.25 grams) which is the same as that for Type 1. This type can be distinguished from Type 1 in the field by its lower habit of growth, but it is extremely difficult to give a morphological explanation for the difference. The chief difference between the two types lies in their period of maturity. In 1911 the plots of Types 1 and 2 were both sown on July 12, but Type 2 was in full flower on September 24, whereas Type 1 did not commence flowering until October 3. Type 2 was ripe on December 5 and Type 1 on December 15. This type was obtained from Bankipur Exhibition in 1910 and has been under observation for two years.

TYPE 3.—“*Safed Bhetmas.*”

Basal portion of stem stout upright, apical portion weak, reclining, secondary branches long, weak, reclining, some tertiaries

ascending. Stems, leaves and pods with tawny pubescence. Flowers purple, 6 to 7 mm. long, sessile in short racemes. Pods 1.3 to 1.7 inches, 1.3 together. Seeds pale yellow, greenish when immature, 4 to 6.5 mm. long by 3 to 4.5 mm. broad, elliptical, flattened. Hilum brown, brown colour extending outwards in a band round the hilum. Germ yellow. Seeds contained 5.4 to 5.9 per cent. nitrogen and 15.5 to 17.5 per cent. oil.

This type and Type 4 are similar except in the colour and composition of the seeds. Their habit is markedly different from that of Type 1. The main stem is usually stout and upright below, but its upper portion and the secondary branches bend down towards the ground. Some of the branches of the higher orders are more ascending. The length of the stem and branches is similar to that of Type 1. In vigorous plants the main stem is erect to a height of about 1 foot and then the main stem droops over, the branches ascend for a few inches and then also droop, their apices lying along the ground. In thickly sown plots the plants grow up straight to a height of about 18 inches and then the stem and two or three long unbranched secondary branches droop down towards the ground. The flowers are concealed beneath the leaves and are not so readily noticeable as in the black types. The reclining stems often root at the nodes. The leaves are flat and somewhat larger than those of Type 1, well developed apical leaflet measuring about 4 inches by 2 inches. The colour of the leaves is also lighter green than in Type 1, but this character is only apparent in the field. The whole plant is tawnily pubescent. The ripening plants when bare of leaves can be distinguished from Type 1 by their brighter yellow colour. This type matures slightly later than Type 1. In 1911 the terminal inflorescences were not produced until November 13, whereas Type 1 had commenced to produce terminal inflorescences on October 27. The pods are ripe by about December 20. This type is usually found growing mixed with Type 4 from which it cannot be distinguished in the field. It was originally obtained from Sankarpur *deanh* near Bhagalpur and has been grown at Sabour

for three years. The brownish pods are slightly curved and from 1—3 seeded. Not more than 3 pods are usually produced on one raceme. The average yield per plant from 500 plants cultivated in the Botanic Experiment Area in 1911 worked out at 49 grams per plant. The weight of 100 seeds varies from 4.8—5.95 grams (mean 5.45 grams).

TYPE 3a.

An impure variety with greenish yellow seeds and a broad brown band round the hilum was obtained from Kalimpong and grown at Sabour in 1911. It was found to be extremely impure, plants differing in having tawny or whitish hairs, stout, short, upright or long twining upright branches and broader or narrower leaves. In the majority of the plants the branches were very numerous, long, thin, upright and twining at the ends. This is variety 1 of Goodwin's list (13), in which it is stated to have the finest flavour of the Darjeeling varieties. It was grown at Sabour in 1911. (Plate III).

TYPE 4.—“*Lal Bhetmas*.”

Basal portion of stem stout, upright, apical portion weak, reclining; secondary branches long, weak, reclining; some tertiaries ascending. Stems, leaves and pods with tawny pubescence. Flowers purple, 6 to 7 mm. long, sessile in short racemes. Pods 1.3 to 1.7 inches, 1.3 together. Seeds chocolate, 4 to 6 mm. long by 3 to 4.5 mm. broad, elliptical slightly flattened only, hilum slightly lighter chocolate. Germ yellow. Seeds contained 5.6 to 6.1 per cent. nitrogen and 15.6 to 17.3 per cent. oil in 1911.

This differs from Type 3 only in its seed characters. The red stains marking injuries to the stem of the type already noticed in the case of Type 1 were also noticed in some plants, and may perhaps be due to the same factor which produces the dark colour of the seed coat in these two types. The average yield per plant of nearly 1,500 plants cultivated in the Botanic area in 1911 worked out at 53 grams per plant. The weight of 100 seeds varies between 4.41 and 6.5 grams (mean 5.53 grams). In field cultivation it appears to be usually mixed with Type 3.

TYPE 4a.

A somewhat similar impure variety has also been obtained from Kalinpong. It has pale chocolate seeds and is listed by Goodwin as variety 2. It differs from the Bengal Type 4 in having seeds of lighter colour, comparatively few long twining branches which lie along the ground, and in ripening considerably earlier. The leaves in some plants were also narrower. The seeds are also heavier than the local variety but have decreased in weight under cultivation in the plains. It was grown at Sabour in 1911.

TYPE 5.—“*Barneli Bhetmas*.”

Stems short, stout, upright; secondary branches upright ascending close to stem. Stems, leaves and pods covered with tawny pubescence. Flowers purple, 6 to 7 mm. long, peduncled on longer racemes. Pods 1 to 7 together. Seeds bright yellow, 4.5 to 7.5 mm. long by 3.5 to 5.5 mm. broad, elliptical, flattened, hilum only brown. Germ yellow. Seeds contained 5.33 per cent. nitrogen and 16.96 per cent. oil in 1911. (1 sample only.)

This type is of erect habit, the stem usually consisting of about 21 nodes and measuring up to 28 inches; the lower branches do not usually exceed 11 inches (8 nodes), but in particularly tall and vigorous plants upright branches of length 31 inches have been noted. The upper branches usually consist of one or two nodes only; the branches ascend close to the main stem. The leaves are large, an apical leaflet measuring 4.8 by 2.7—5 inches and are covered with longer and more upright hairs than in other types. The plot sown at Sabour on July 12, 1911, was in flower on September 24 and some plants were ripe on November 18, though the bulk of the crop was harvested on December 9. The flowers are produced in rather elongated racemes on which from 1 to 7 pods are produced. The ripe pods are whitish and covered with pale yellow hairs. The crop when dead ripe has a distinct whitish appearance. The weight of seed per plant produced by 20 good average plants was at the rate of 42 grams per plant, whereas that for 100 average plants was at the rate of 20 grams per plant. The weight of 100 seeds was 8.25 grams when

growing at Kalimpong, but fell to 7.4 after cultivation at Sabour.

This type is grown at Kalimpong and was grown at Sabour and on all the Bengal farms in 1911. The variety was listed as variety 4 by Goodwin (13) in the Report of the Kalimpong Farm for 1910-11. Commonly a weak plant with thinner prostrate or partially ascending stems and smaller leaves, but similar as regards period of maturity, shortness of nodes, pubescence, and seed colour, is found in this crop. (Plate II).

TYPE 6.—“*Nepali Bhelmas.*”

Stems short, stout, upright; secondary branches short, upright ascending close to stem. Leaves nearly glabrous, stems and pods covered with tawny pubescence. Flowers white, 6 to 7 mm. long, sessile in short racemes. Pods 1 to 3 together. Seeds brown, 8 to 10 mm. long, by 6 to 8 mm. broad, oval not flattened, germ yellow. Seeds contained 5.72 per cent. nitrogen and 20.04 per cent. oil.

This type has a short, stout, upright stem varying in length from 6 to 15 inches, the branches are few and short, the lower branches not exceeding 6 inches in length and the upper being considerably shorter; they make an angle of less than 30° with the main stem. The stems are brownish and covered with tawny pubescence. The leaves are flat, covered with closely adpressed hairs and dark green in colour, they vary in size from 3 inches by 1.8 inches to 5 inches by 2 inches broad. The leaflets are deciduous from the upright petioles. The plot planted at Sabour on July 12, 1911, was in flower on September 6 and was ripe by October 30. The pods are brownish and covered with tawny pubescence. Sixty-five plants of the general crop of this variety in 1911 produced seed at the rate of 5 grams per plant only. The seed grown at Kalimpong weighed 24.4 grams per 100 seeds, while that grown at Sabour was shrivelled and pitted and only weighed 12.8 grams per 100.

This type was obtained from Kalimpong and was tested at Sabour in 1911. It is listed as variety 6 by Goodwin in the

Report of the Kalimpong Farm for 1910-11, where it is stated that this variety is cultivated at higher altitudes than the other varieties. It does not do so well when sown as a mixed crop with maize as when grown by itself. (Plate III).

5. DISTRIBUTION.

Decandolle (14) came to the conclusion that soy beans are a modern introduction into India, because Roxburgh had only seen an introduced plant in the Calcutta Botanic Gardens. Our observations show that the black, chocolate, and yellow seeded varieties of soy beans (Types I—IV) are cultivated under the name Bhetmas to a very limited extent indeed in the plains of Bengal. The only plots which we have actually seen growing have been in the neighbourhood of Sabour from seed stolen from the Botanical area. Samples of the seeds have, however, been met with at exhibitions in Bihar, Orissa and Chota Nagpur. In the Sonthal Parganas the yellow and chocolate varieties are cultivated with cotton and are called Disombhorac. It is, therefore, possible that soy beans may have been cultivated in the plains before Roxburgh's time, but escaped his notice owing to the extremely limited extent of their cultivation. In the hills the following five varieties, black seed (Type 1A), greeny yellow (Type IIIA), pale chocolate (Type IVA), Barmeli and Nepali, are cultivated to a small extent and of these the Barmeli variety would appear to be the commonest. Soy beans can be cultivated up to an elevation of 6,000 feet where *Phaseolus* Spp. will not thrive.

With regard to their distribution it is interesting to note that all the varieties cultivated in the plains are pure, whereas those cultivated in the hills are usually very impure. The types present in the plains are all late varieties with long twining branches, tawny hairs and purple flowers, whereas the Darjeeling varieties appear to contain plants exhibiting many other characters such as upright habit, white flowers, adpressed hairs on the upper leaf surface, white pubescence and various seed characters which are present in the Chinese varieties introduced into America. The time of maturity of the Darjeeling varieties also

varies. These facts make it seem probable that the Darjeeling varieties were originally obtained from China through Tibet, and have spread thence into the plains, where only these types best adapted to the plains have survived.

Shaw (10) states that in China "the further north they extend the better in quality do the beans become." Our observations show that the seed of a variety cultivated in the Himalayas is heavier than the seed of the same variety cultivated in the plains. It is probable therefore that soy beans find more suitable conditions in the hills than the plains, and this would account for their having spread outwards for a short distance only from the foot of the hills. We cannot therefore expect to obtain much success in acclimatizing soy beans, except along the foot of the Himalayas and our greatest chance of introducing the crop successfully will be in the Himalayas.

6. CULTIVATION.

In the plains soy beans can be grown alone or with maize, or cotton. They will grow satisfactorily on high lands and do not necessarily require manure. The land should receive some 3 or 4 ploughings and should be clean and in good tilth. The seed may be sown broadcast at the rate of 10 seers or in drills two to three feet apart according to variety at 6 seers per acre from the break of the monsoon to the end of July, though a smaller crop could be obtained from seed sown up till the middle of September. Shaw (10) states that in China the seed is sown by hand on top of drills and covered by hand. The crop is benefited by one or two hoeings in July and August, but after that will require no further treatment until harvest, though a hoeing in September after the close of monsoon is useful to conserve the moisture. It is probable that the crop could also be sown broadcast at a slightly greater seed rate in September on *Kulthi* and *Kalai* lands to take the place of these crops. The late varieties (Types I—IV) flower in October and their yield will be considerably decreased if the land dries before that time. The plants will be ready for harvest after the middle of December and should be harvested before they are fully ripe as the

seed sheds easily. The late varieties at present cultivated in the plains are unsatisfactory, in that their twining habit makes intercultivation difficult and their long growing period prevents the ground from being used for two crops in the year, besides which they harbour rats during October to December.

The usual method of cultivation of soy beans in the Darjeeling hills is described by Goodwin (13) as follows :—"After the maize crop has received the second hoeing and the weeds have been carefully buried the seed is sown among it at the rate of 40 lbs. per acre in June or the beginning of July. After the maize has been harvested the top parts of the stalks are cut off and carried away, and the Bhetuas crop is then hand weeded. The weeds may be pulled up and laid on the ground if the crop is not very thick, but they are often tied on to the maize stalks which have been left standing. After weeding the crop soon covers the ground with a mass of luxuriant vegetation, the appearance of which is very fine. Soy bean ripens in November. The leaves usually fall off before the crop is harvested and add to the manurial residue left by the roots, the nodules of which are very conspicuous." Goodwin has found that the Nepali variety does not do well if grown with maize, but sown by itself in drills 1 foot apart in April, it yielded 26 mds. (approx. 1 ton) per acre. Soy beans are also grown along the banks round the paddy fields in Kalimpong.

7. YIELD.

The tables given below show the actual yields obtained in experiments carried out in Bengal during the past year on unmanured land. The results of the experiments at Chinsurah and Cuttack have been supplied by Mr. F. Smith, and those of the Sabour, Bankipur and Dumraon farms by Mr. G. Sherrard, while all the information concerning the cultivation of soy beans in Kalimpong has been obtained from Mr. P. Goodwin. It will be seen that the yield usually varies from 8—12 mds. (approximately 650—1,000lbs.), but under favourable circumstances it may go up to over 26 mds. (over 2,200lbs.) per acre. It may be noted that these yields are much superior to those previously

Yield of the Types of Soy Beans found in Bengal, Bihar and Orissa.

Variety.	Where grown.	Dates of sowing.	Area sown.	Approximate yield per acre in lbs.	Corresponding yield in Mds. Srs. & Chks.	REMARKS.
Type 1.—Black seed. The black seed was sown from Bankipur Division and gave a very poor germination.	Sabour B. E. A. Farm Bankipur Farm, A II Dumraon Farm, I Chinsura Farm I " " II Outtaek Kallimpong Farm	June 7, 1911 July 9, 1911 May 31, 1911 June 29, 1911 July 10, 1911 June 10, 1911 June 5, 1911 June 12, 1911 1911	0.22 acre 1.0 " " 0.1 " " 0.5 " " 0.5 " " 0.31 " " 0.2 " " 0.2 " " 1.0 "	482 642 1,100 (ton approx.) 333 263 710 930 452 382	Mds. Srs. Ch. 2 23 0 7 23 0 13 20 0 4 0 0 3 8 0 10 27 0 10 27 0 5 20 0 4 26 0	Germination poor. Water-logged. Heavy low land. Clay loam. Loam. From 2 to 2 1/2 acre. With Maize.
Type 1-A.—Yellow	Sabour B. E. A. Farm " " Farm Chinsura Farm I " " II Kallimpong Farm	June 7, 1911 June 9, 1911 June 10, 1911 July 1, 1911 1911	.19 1.1 0.31 0.2 1.22	903 888 700 1,068 1,308	10 39 0 10 32 0 8 20 0 12 10 0 15 36 0	Water-logged. Clay loam. Loam. With Maize.
Type 3-A.—Greeny yellow Kallimpong Farm. (Kallimpong).	Sabour B. E. A. Farm Bankipur Farm, A I Dumraon Farm Outtaek Farm	June 7, 1911 July 9, 1911 May 31, 1911 June 29, 1911 June 10, 1911 June 12, 1911	0.33 1.0 1.1 1.5 0.3 0.2	1,102 874 2,161 802 463 823	13 16 0 9 20 0 26 12 0 8 30 0 0 0 0 10 0 0	Heavy low land. mds. 10 srs. were obtained in a second plot 0.2 acre at Outtaek.
Type 4.—Chocolate	Kallimpong Farm	1911	.5	806	9 23 0	With Maize.
Type 4-A.—Pale chocolate (Kallimpong).	Kallimpong Farm Chinsura Farm I " " II Kallimpong " II	June 1911 June 1911 July, 1911 1911 5 acres. 2.0 acres.	1,226 1,028 1,398 880	14 35 0 12 20 0 6 31 0 16 24 0	Clay loam. Germ poor, loam.
Type 6.—Nepali	Kallimpong	April 1911 1910	.25 .25	2,189 211	20 28 0 2 24 0	With Maize.

NOTE.—These yields were obtained on unmanured land.

obtained in India (10). In China (12) the yield is said to vary from 400—600lbs. per acre in the poorer districts along the Yellow Sea coasts up to 2,000lbs. per acre in the Tichling district.

The cost of cultivation to produce a crop of 8—12 maunds should not be greater than Rs. 10—15 per acre in the plains, though it may be increased to Rs. 20 per acre if many weedings are given. One or at most two weedings are probably sufficient if the crop has germinated well. At Kalimpong, Goodwin (13) produced a crop of 17 maunds 12 seers (approximately 1,420lbs.) grain at a cost of Rs. 22 annas 8 for cultivation (excluding rent).

8. PRICE.

Although experiments are being made all over the world on the possibility of establishing soy beans as a paying crop, yet it would appear that Manchuria is still the only source from which they are obtained in large quantities.

Up till recent years the prices of Manchurian soy beans have been so small that it was unlikely to pay to grow the crop in other parts of the world. There is, however, an indication that the prices are steadily rising and, in fact, while in the autumn of 1908 the price was only £4.15 in England it went up to as much as £8.5 per ton towards the end of 1910 (Shaw 10). At the same time the price of oil went up to £30 per ton and the soja meal, obtained after extracting the oil, was sold at about £6.10* per ton.

Now by purchasing and selling at these rates, the manufacturer would have paid for 100 tons, £825. It may be assumed that some 13 tons of oil and 87 tons of meal could have been obtained from this which would have sold for £390 and £565.10 respectively. His gross receipts therefore on 100 tons, at these rates, would have been £955.10, and his gross profits about £130. This would have allowed £1.6 per ton for cost of manufacture and profit, which would be equivalent

* We have recently received a letter from England quoting the price of coarse meal at £7.5 and fine meal at £7.6 per ton from May to August 1912. We are indebted for this information to the courtesy of the Hull Oil Manufacturing Company, Limited.

to about 16% of the cost price. This is not sufficient margin to allow us to assume that higher prices could be given for the raw material, unless an increased price were obtained either for the oil or the meal.

It would appear, however, that £6-10 per ton is a ridiculously low price to pay for a food of such concentrated feeding quality as soy bean meal. The former cheapness of the meal has been due probably to the fact, that until quite recently it was found impossible to remove the objectionable odour due to the solvents used in extracting the oil. Of late, however, a meal has been put on the market which has not any trace of smell of the solvents used, and it is possible that the advent of this meal will raise the price of the product to a considerable extent, and enable the manufacturer to pay better prices for the raw material.

As prices are at present, it would appear doubtful whether the growth of the soy bean crop could be extended for export purposes. Our experiments appear to point out that yields of 8-12 maunds per acre may be expected with reasonable care, and at the present time prices of Rs. 3/- to 3/4 per maund free on rail is required by the few growers of indigenous soy beans. This works out at about £5-8 to £5-17 per ton. The reason for this high rate is not that the cost of cultivation is high as it is probable that in Bihar it should not exceed Rs. 10/- to 15/- per acre. The crop, however, is a difficult one in that it is on the land much longer than other pulses, and that it has in consequence to pay rent for two seasons. This difficulty may be met by the introduction of early ripening varieties, but of this we are unable to speak with certainty. Unless a higher yield or an earlier ripening variety can be obtained, however, there would appear to be little possibility of making sufficient profit at the price of Rs. 2-8 per maund or £4-10 per ton which is offered by merchants in Calcutta.

There can be no doubt that the crop is intrinsically worth more than this, for it has been shown that, with reasonable prices for the oil and meal, the manufacturer can obtain over £9-10 per ton for the seed after manufacture.

Also it has been pointed out already that the present price of £7-5 per ton for the meal is only for meal as cattle food. If success crowns the recent attempts to put the meal on the market for biscuit and cake manufacture it is evident that the manufacturer will ask for more than £7-5 per ton for the fine meal.

With the price of the coarsest wheat flour at £10 to £11 per ton it may be assumed that the manufacturer will be justified in asking for soy bean flour, which is much more nutritious, at least £8 to £10 per ton. With the price at the lower figure from 100 tons of seed the manufacturer would get 87 tons of meal worth £696 and 13 tons of oil worth £390 or £1,086 worth of manufactured products. In this way he would get nearly £11 per ton of seed which should allow him easily to pay the £9 per ton in England required to bring the price up to £7 per ton or Rs. 4 per maund in Calcutta.

It will, therefore, be seen from what precedes that if the growth of soy beans is to spread in India we must obtain either :—

(a) Higher prices from the manufacturers.

(b) Higher yields of seed per acre and earlier ripening varieties.

It would appear that neither of these conditions is impossible, but it is uncertain whether either will be fulfilled.

9. USES.

Food-stuff :—In Bengal soy beans are used very little for food as they are said to be too heat producing. It is usually taken after frying over a heated sand bath as *bhunja*, but it is also heated, crushed, and then used as *dal*, and also as *larua* mixed with *gur*.

The high percentage of nitrogen and oil in this pulse should make it a particularly valuable addition to a rice diet as a preventative of *beri-beri*. That there are a number of other ways of preparing soy beans for food in addition to those mentioned above can be seen from the following extracts from Shaw's Soy Bean in Manchuria (10) which are quoted here at length for the benefit of those desirous of experimenting with soy beans as an article of diet,

"In the Far East the beans are put to a variety of uses :—
 (a) As a food-stuff they are made into—

I. Bean sauce, or soy, called in Japan "*shoyu*" (whence the name "soya") and in China, *chiang-yu*. It is made by boiling the beans, adding an equal quantity of wheat or barley, and leaving the mass to ferment : a layer of salt and three times as much water as beans are afterwards put in, when the liquid is pressed and strained. The following method is more scientific and cleanly than the native way :—Equal weights of beans, coarse barley meal, and salt should be taken, the beans washed and boiled until tender, and pounded in a mortar, the barley meal being added gradually. The mass should then be placed in an earthenware bowl and covered with a cloth, to be stood in a warm place for several days, until it is sufficiently fermented, but not mouldy. The salt is then dissolved in water and stirred into the mass, which must be kept closely covered for three months, during which time it must be stirred for one hour daily. At the end of this time it is strained through a fine cloth, as much of the moisture as possible being extracted by pressure ; after it has become clear the sauce is ready for use.

II. The Chinese paste *chiang*—not the same article of diet as the Japanese paste *miso*. It is made by farmers, and eaten with fish, meat, and vegetables, while the more expensive Chinese soy is only made by wealthy families and restaurant-keepers and is not consumed by the very poor.

There are two kinds of *chiang* : *ta* (great) and *hsiao* (small). The former is made in the following way :—The ingredients—1 *ton* of yellow beans and 2 *ton* of water—are boiled until the mass of the former can be readily crumbled in the fingers. It is next ground with a wooden pestle in a tub into the shape of a pancake, and laid on a mat in a cool place for fermentation, which is complete at the end of two months. The fermented mass is then re-ground to powder and 4 *sheng* (native pints) of salt with water are added, after which it is placed in the open and stirred occasionally. As the water evaporates more must be added and in 15 days the mixture is ready for consumption.

Hsiao chiang is made in the same way, but the ingredients are 1 *tou* 1 *sheng* (1 peck and 1 pint) of beans to 1 *sheng* of maize, to which 5 *sheng* of salt are added.

III. *Tou-fu*, or beancurd—made from green or yellow beans, the first giving greater quantity but poorer quality. The beans are steeped in water until they swell considerably, then ground in a stone mill, and passed through a strainer which retains the epidermis of the beans; after which they are boiled in a pot, poured off into a jar, and well diluted brine added to them—this being stirred in, causes coagulation of the proteid compound legumin or vegetable casein, and the mixture is ready to be drained off in an hour and cut into blocks for sale."

Some trials of the value of soy beans as a green vegetable carried out by Mr. Sil at Sabour showed that the pods were unpleasantly fibrous.

Oil.—The chief use of the oil is for soap-making, and it is for the extraction of the oil for this purpose that the seeds are being imported into England on a large scale. In China (13) the oil is used as an illuminant, as a substitute for lard in cooking, though it is inferior to rapeseed and sesamum oil for this purpose, as a lubricant for greasing cart axles, and for water-proofing cloth.

Cake and Flour.—The flour is already utilized for the manufacture of biscuits and should prove a valuable food-stuff as it contains a high percentage of nitrogen. The bean cake has given results equal to decorticated cotton cake in a number of feeding trials with milch cows (15).

10. GENERAL CONCLUSIONS.

At the present time Soy Beans are grown to a slight extent only in the Darjeeling hills and to no appreciable extent elsewhere although satisfactory yields have been obtained in the experiments conducted by the Agricultural Department in both these areas. We may ascribe the present unpopularity of the crop to the following reasons. For export the price offered in Calcutta is not yet sufficiently attractive; as a food-stuff it is

more potent than the ordinary pulses to which the people are accustomed; as a crop for growth in the plains it has the disadvantage of occupying the land during two seasons, it may suffer from water-logging during the rains and requires plenty of moisture in October, and it harbours rats during the last two months of its growth. These objections do not appear to us by any means unsurmountable. We have already shown that there are good prospects of an increase of price of Soy Bean meal, which should enable manufacturers to pay more for their raw material. The use of Soy Beans for food could be extended if the educated classes once appreciate its value as an addition to a rice diet, and experiment with its preparation for food on the lines suggested. As a field crop in the plains it can suitably replace *Urd* or *Kalai* (*Phaseolus Mungo*, Linn) as a mixed crop with Maize, in which case the maize would be harvested in September and the Soy Beans in December. It could also take the place of the *Kulthi* (*Dolichos biflorus*) and sometimes *Kalai*, which are sown on large areas of high lands in September and which do not give very heavy or profitable crops. Its cultivation in the hills would probably be largely extended on the present lines as soon as the price reached a satisfactory figure. It only remains to say that our work is being continued on the lines indicated in this paper with a view to isolating early maturing types possessing a high yield of oil.

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BLACK SOY BEANS.

TABLE I.

Table showing the history of descendants of various singly selected plants.

		1900.		1910.		1911.		OIL.		REMARKS.
		No. of plants,	Nitrogen.	No. of plants,	Nitrogen.	No. of plants,	Nitrogen.	1910.	1911.	
135 A	...	1	6.08	9	6.27	103	6.58	...	12.36	Type 1.
135 B	...	1	5.92	6	6.58	49	6.09	...	13.34	
135 C	...	1	6.43	4	6.77	65	6.20	...	12.22	
E ₀ /26	1	7.49	6	6.27	14.30	12.24	
E ₁ 35	1	6.11	5	6.55	15.38	11.18	These were selected in 1910 for abnormalities in nitrogen and oil contents but their descendants were found to be normal.
E ₀ /39	1	6.10	53	6.22	14.22	13.50	
Y ₀ /65	1	5.95	47	6.40	14.86	13.14	Type 2. These were selected in 1910. They appeared to ripen early and gave small seeds. The nitrogen contents were also very high. Their descendants have been found to return to the normal in this particular. The oil contents are also normal.
256 A	1	7.44	36	6.05	...	12.84	
256 B	1	7.23	6	6.11	...	13.34	
256 C	1	7.70	8	6.52	...	12.66	
256 D	1	6.87	6	6.43	...	12.42	
256 F	1	6.66	55	6.54	...	12.48	
256 G	1	7.44	16	6.24	...	13.36	
256 H	1	6.74	32	6.08	...	11.27	
256 (bulkcd)	23	7.04	59	6.32	...	13.42	

YELLOW SOY BEANS (TYPE 3).

TABLE II.

Table showing history of descendants of various singly selected plants.

Original No.	1909.		1910.		1911.		OIL.		REMARKS.
	No. of plants.	Nitrogen.	No. of plants.	Nitrogen.	No. of plants.	Nitrogen.	1910.	1911.	
95 A ...	1	5.90	2	5.67	186	5.83	...	16.30	These were selected in 1910 as being abnormal in nitrogen or oil content. In every case their descendants have shown a tendency to revert to the mean.
95 B ...	1	5.93	21	5.79	182	5.52	...	15.95	
95 C ...	1	5.60	6	5.65	117	5.77	...	16.90	
E ₂ /53	1	6.14	32	5.96	16.38	15.48	
E ₂ /55	1	6.08	9	5.37	18.14	17.52	
E ₂ /60	1	6.10	18	5.87	15.48	16.88	
E ₂ /77	1	6.11	9	5.41	17.22	16.19	
E ₂ /83	1	4.80	4	5.73	19.06	17.32	
E ₂ /86	1	5.47	5	5.48	17.76	17.28	
E ₂ /101	1	6.18	4	5.81	16.12	16.48	
E ₂ /131	1	5.01	4	5.77	17.06	16.36	
E ₂ /145	1	4.90	14	5.65	19.32	16.50	

CHOCOLATE SOY BEANS (TYPE 4).

TABLE III.

Table showing history of descendants of various singly selected plants.

Original No.	1909.		1910.		1911.		OIL.		REMARKS.
	No. of plants.	Nitrogen.	No. of plants.	Nitrogen.	No. of plants.	Nitrogen.	1910.	1911.	
94 A ...	1	5.32	2	5.91	180	6.06	16.44	For analyses of the progeny of each of the 21 single plants in 1911, see Table IV.
94 B ...	1	5.44	19	5.85	306	5.93	17.16	
94 C ...	1	4.83	24	5.80	1,227	6.01	16.09	
E2/1	1	4.93	6	5.60	17.62	15.56	These were selected in 1910 as having nitrogen content below normal and oil above normal. All show a tendency to revert to the normal in both cases.
E2/3	1	4.75	48	5.89	18.22	17.28	
E2/5	1	4.73	43	5.45	19.16	18.10	
E2/82	1	4.83	23	5.74	19.30	16.66	
E2/83	1	5.07	37	5.96	19.44	17.34	
E2/86	1	4.93	14	5.67	19.38	16.08	

TABLE IV.

Table showing variations in 24 plots grown from each plant descended from sowing of seed from the single plant No. 94c.

Serial No.	No. of plants.	Nitrogen.	Variation from mean.	Oil.	Variation from mean.	REMARKS.
1	95	5.82	-.19	16.94	+.91	
2	48	6.05	+.04	17.30	+1.27	
3	62	6.06	+.05	15.80	-.21	
4	46	5.58	-.43	15.56	-.45	
5	41	6.07	+.06	15.38	-.63	
6	87	6.00	-.01	15.26	-.75	
7	123	6.18	+.17	16.06	+.65	
8	33	6.02	+.01	16.28	+.27	
9	31	6.11	+.10	15.20	-.81	
10	26	6.15	+.14	16.30	+.20	
11	59	6.09	+.08	15.90	-.11	
12	61	5.74	-.27	15.12	-.89	
13	84	5.94	-.17	14.84	-1.17	
14	68	6.11	+.10	15.98	-.03	
15	32	5.82	-.19	15.86	-.15	
16	43	5.95	-.07	15.96	-.05	
17	40	5.87	-.14	15.84	-.17	
E-58	18	6.14	+.13	16.40	+.39	
E-59	17	6.00	-.01	16.10	+.09	
E-60	1	6.16	-.15	16.68	+.67	
	70	6.04	+.03	16.20	+.19	
	22	6.22	+.21	15.31	-.67	
	23	6.07	+.06	17.58	+1.57	
	24	5.98	-.03	16.36	+.35	
Mean	...	6.01	...	16.03	..	

It will be seen from this Table that the variations are very small. In the case of the nitrogen determinations 13 are within the limits $6.01 \pm .1$ making the probable error only $\pm .1$ and in the oils 13 lie within the limits $16.03 \pm .5\%$ pointing to a probable error of $\pm .5\%$.

TABLE V.

Detailed table showing the results obtained with 150 samples of black soy beans analysed in 1911.

No.	Nitrogen Percentage.	Oil Percentage.	Oil Percentage on the dry substance.	REMARKS.
E-1 P-1 of 10 ...	6.80	This was determined in certain cases but was found to make no difference to our conclusions. In consequence the percentage of oil and nitrogen on undried substance has been inserted in the letterpress.	
E-1 P-2 ...	6.68		
E-1 P-3 ...	7.54	14.26		
E-1 P-4 ...	6.50		
E-1 P-5 ...	Spoilt		
E-1 P-6 ...	6.54		
E-1 P-7 ...	6.90		
E-1 P-8 ...	6.23		
E-1 P-9 ...	6.25		
E-1 P-10 ...	6.20		
E-1 P-11 ...	7.66	14.24		
E-1 P-12 ...	7.03		
E-1 P-13 ...	7.05	13.32	15.45	
E-1 P-14 ...	6.92	
E-1 P-15 ...	7.34	13.92	

TABLE V—*contd.*

Nitrogen percentage.	Oil percentage.	Oil percentage on the dry substance.	REMARKS.
6.63	13.86	16.14	
7.07	
6.61	
6.86	13.38	15.50	
6.80	13.62	15.80	
7.30	13.96	
7.19	13.28	15.02	
7.67	11.76	12.72	
7.01	
6.57	13.50	15.18	
7.40	14.30	
6.97	14.38	
7.00	13.88	
7.29	
6.22	
6.69	

TABLE V—*contd.*

No.	Nitrogen percentage.	Oil percentage.	Oil percentage on the dry substance.	REMARKS.
$\frac{E-1}{P-32}$...	6.58	
$\frac{E-1}{P-33}$...	6.51	
$\frac{E-1}{P-34}$...	7.35	14.06	
$\frac{E-1}{P-35}$...	6.11	15.38	
$\frac{E-1}{P-36}$...	6.41	
$\frac{E-1}{P-37}$...	7.12	
$\frac{E-1}{P-38}$...	6.61	
$\frac{E-1}{P-39}$...	6.10	14.22	
$\frac{E-1}{P-40}$...	6.55	
$\frac{E-1}{P-41}$...	6.29	
$\frac{E-1}{42}$...	6.43	15.10	
$\frac{E-1}{43}$...	6.20	14.22	
$\frac{E-1}{44}$...	6.61	13.94	
$\frac{E-1}{45}$...	7.28	12.60	
$\frac{E-1}{46}$...	6.55	16.28	
$\frac{E-1}{47}$...	6.78	12.82	

TABLE V—*contd.*

No.	Nitrogen percentage.	Oil percentage.	Oil percentage on the dry substance.	REMARKS.
$\frac{E-1}{48}$...	6.75	12.76	
$\frac{E-1}{49}$...	6.46	13.54	
$\frac{E-1}{50}$...	7.09	14.02	
$\frac{E-1}{51}$...	6.43	13.04	
$\frac{E-1}{52}$...	7.03	15.08	
$\frac{E-1}{53}$...	6.23	15.24	
$\frac{E-1}{54}$...	6.48	12.40	
$\frac{E-1}{55}$...	6.33	14.56	
$\frac{E-1}{56}$...	Spoilt	13.50	
$\frac{E-1}{57}$...	6.71	11.74	
$\frac{E-1}{58}$...	5.86	13.66	
$\frac{E-1}{59}$...	6.11	15.06	
$\frac{E-1}{60}$...	6.32	13.52	
$\frac{E-1}{61}$...	6.25	13.02	
$\frac{E-1}{62}$...	6.25	14.06	
$\frac{E-1}{63}$...	6.25	13.62	

TABLE V—*contd.*

No.	Nitrogen percentage.	Oil percentage.	Oil percentage on the dry substance.	REMARKS.
$\frac{E-1}{P-32}$...	6.58	
$\frac{E-1}{P-33}$...	6.51	
$\frac{E-1}{P-34}$...	7.35	14.06	
$\frac{E-1}{P-35}$...	6.11	15.38	
$\frac{E-1}{P-36}$...	6.41	
$\frac{E-1}{P-37}$...	7.12	
$\frac{E-1}{P-38}$...	6.61	
$\frac{E-1}{P-39}$...	6.10	14.22	
$\frac{E-1}{P-40}$...	6.55	
$\frac{E-1}{P-41}$...	6.29	
$\frac{E-1}{42}$...	6.43	15.10	
$\frac{E-1}{43}$...	6.20	14.22	
$\frac{E-1}{44}$...	6.61	13.94	
$\frac{E-1}{45}$...	7.28	12.60	
$\frac{E-1}{46}$...	6.55	16.28	
$\frac{E-1}{47}$...	6.78	12.82	

TABLE V—*contd.*

No.	Nitrogen percentage.	Oil percentage.	Oil percentage on the dry substance.	REMARKS.
$\frac{E-1}{48}$...	6.75	12.76	
$\frac{E-1}{49}$...	6.46	13.54	
$\frac{E-1}{50}$...	7.09	14.02	
$\frac{E-1}{51}$...	6.43	13.04	
$\frac{E-1}{52}$...	7.03	15.08	
$\frac{E-1}{53}$...	6.23	15.24	
$\frac{E-1}{54}$...	6.48	12.40	
$\frac{E-1}{55}$...	6.33	14.56	
$\frac{E-1}{56}$...	Spoilt	13.50	
$\frac{E-1}{57}$...	6.71	11.74	
$\frac{E-1}{58}$...	5.86	13.66	
$\frac{E-1}{59}$...	6.11	15.06	
$\frac{E-1}{60}$...	6.32	13.52	
$\frac{E-1}{61}$...	6.25	13.02	
$\frac{E-1}{62}$...	6.25	14.06	
$\frac{E-1}{63}$...	6.25	13.62	

TABLE V—*contd.*

No.	Nitrogen percentage.	Oil percentage.	Oil percentage on the dry substance.	REMARKS.
$\frac{E-1}{64}$...	6.66	13.62	
$\frac{E-1}{65}$...	5.95	14.86	
$\frac{E-1}{66}$...	6.76	13.48	
$\frac{E-1}{67}$...	7.03	13.10	
$\frac{E-1}{68}$...	6.73	12.82	
$\frac{E-1}{69}$...	6.87	13.30	
$\frac{E-1}{70}$...	6.53	14.02	
$\frac{E-1}{71}$...	6.37	12.88	
$\frac{E-1}{72}$...	7.19	13.33	
$\frac{E-1}{73}$...	7.06	12.85	14.69	
$\frac{E-1}{74}$...	6.46	13.20	
$\frac{E-1}{75}$...	6.46	13.58	
$\frac{E-1}{76}$...	6.74	12.96	
$\frac{E-1}{77}$...	Spoilt	13.14	15.24	
$\frac{E-1}{78}$...	6.21	13.28	15.50	
$\frac{E-1}{79}$	13.48	15.50	

TABLE V—*contd.*

No.	Nitrogen percentage.	Oil percentage.	Oil percentage on the dry substance.	REMARKS.
$\frac{E-1}{80}$...	6.61	14.45	16.30	
$\frac{E-1}{81}$...	6.50	14.16	15.95	
$\frac{E-1}{82}$...	6.49	11.34	
$\frac{E-1}{83}$...	6.76	13.18	14.30	
$\frac{E-1}{84}$...	6.61	
$\frac{E-1}{85}$...	6.58	11.12	13.02	
$\frac{E-1}{86}$...	6.14	12.78	14.80	
$\frac{E-1}{87}$...	6.07	15.44	
$\frac{E-1}{88}$	
$\frac{E-1}{89}$...	6.55	
$\frac{E-1}{90}$...	6.78	10.48	11.70	
$\frac{E-1}{91}$...	6.79	
$\frac{E-1}{92}$	
$\frac{E-1}{93}$...	6.62	
$\frac{E-1}{94}$...	7.07	
$\frac{E-1}{95}$...	6.50	



TABLE V—*contd.*

No.	Nitrogen percentage.	Oil percentage.	Oil percentage on the dry substance.	REMARKS.
$\frac{E-1}{96}$...	6.81	11.65	13.77	
$\frac{E-1}{97}$...	6.72	14.06	16.40	
$\frac{E-1}{98}$...	6.60	
$\frac{E-1}{99}$...	6.46	12.82	14.70	
$\frac{E-1}{100}$...	6.52	11.90	13.75	
$\frac{E-1}{101}$...	6.28	14.00	16.20	
$\frac{E-1}{102}$...	6.37	
$\frac{E-1}{103}$...	7.12	
$\frac{E-1}{104}$...	7.04	
$\frac{E-1}{105}$...	6.34	13.43	15.55	
$\frac{E-1}{106}$...	6.33	
$\frac{E-1}{107}$...	Spoilt.	
$\frac{E-1}{108}$...	7.18	12.98	15.08	
$\frac{E-1}{109}$..	6.60	
$\frac{E-1}{110}$...	6.69	
$\frac{E-1}{111}$...	6.99	12.42	14.40	

TABLE V—*contd.*

No.		Nitrogen percentage.	Oil percentage.	Oil percentage on the dry substance.	REMARKS.
$\frac{E-1}{112}$...	7.00	13.42	15.40	
$\frac{E-1}{113}$...	7.05	
$\frac{E-1}{114}$...	6.47	
$\frac{E-1}{115}$...	7.01	
$\frac{E-1}{116}$...	7.14	
$\frac{E-1}{117}$...	7.03	13.38	15.52	
$\frac{E-1}{118}$	
$\frac{E-1}{119}$	14.16	16.20	
$\frac{E-1}{120}$...	7.34	
$\frac{E-1}{121}$...	7.13	
$\frac{E-1}{122}$...	6.82	
$\frac{E-1}{123}$...	7.37	15.82	
$\frac{E-1}{124}$...	6.80	
$\frac{E-1}{125}$...	6.72	
$\frac{E-1}{126}$...	6.96	
$\frac{E-1}{127}$...	7.09	

TABLE V—*contd.*

No.	Nitrogen percentage.	Oil percentage.	Oil percentage on the dry substance.	REMARKS.
$\frac{E-1}{128}$...	6.19	
$\frac{E-1}{129}$...	6.69	
$\frac{E-1}{130}$...	6.15	
$\frac{E-1}{131}$...	6.17	
$\frac{E-1}{132}$...	6.94	
$\frac{E-1}{133}$...	6.30	
$\frac{E-1}{134}$...	6.15	
$\frac{E-1}{135}$...	7.32	
$\frac{E-1}{136}$...	6.47	
$\frac{E-1}{137}$...	6.86	
$\frac{E-1}{138}$...	7.65	
$\frac{E-1}{139}$...	7.30	13.02	
$\frac{E-1}{140}$...	7.36	12.06	...	
$\frac{E-1}{141}$...	6.69	
$\frac{E-1}{142}$...	6.28	
$\frac{E-1}{143}$...	6.96	

TABLE V—*concl'd.*

No.	Nitrogen percentage.	Oil percentage.	Oil percentage on the dry substance.	REMARKS.
$\frac{E-1}{144}$...	6.48	
$\frac{E-1}{145}$...	7.20	
$\frac{E-1}{146}$...	6.93	
$\frac{E-1}{147}$...	6.82	
$\frac{E-1}{148}$...	6.69	
$\frac{E-1}{149}$...	6.58	
$\frac{E-1}{150}$...	6.61	

TABLE VI.

Detailed results obtained in 1911 with 150 samples of yellow soy beans (Type 3).

No.	Nitrogen percentage.	Oil percentage.	Oil percentage on the dry substance.	REMARKS.
$\frac{E-3}{1}$...	5.78	17.30	19.60	The oil content in the dry substance was calculated but was found to make no difference to our conclusions. In consequence, the actual percentages observed on the undried substance have been used in the letterpress.
$\frac{E-3}{2}$...	5.82	16.58	18.78	
$\frac{E-3}{3}$...	5.99	17.34	19.57	
$\frac{E-3}{4}$...	5.46	
$\frac{E-3}{5}$...	5.60	16.64	19.04	

TABLE VI—*contd.*

No.	Nitrogen percentage	Oil percentage.	Oil percentage on the dry substance.	REMARKS.
$\frac{E-3}{6}$...	5.52	17.22	19.68	The oil content in the dry substance was calculated but was found to make no difference to our conclusions. In consequence the actual percentages observed on the undried substance have been used in the letter-press.
$\frac{E-3}{7}$...	5.76	16.98	19.38	
$\frac{E-3}{8}$...	5.34	16.84	19.29	
$\frac{E-3}{9}$...	5.76	16.78	18.95	
$\frac{E-3}{10}$...	5.53	
$\frac{E-3}{11}$...	5.74	16.60	18.72	
$\frac{E-3}{12}$...	5.76	
$\frac{E-3}{13}$...	5.44	17.06	19.17	
$\frac{E-3}{14}$...	5.61	
$\frac{E-3}{15}$...	5.70	17.25	19.66	
$\frac{E-3}{16}$...	5.57	17.38	19.84	
$\frac{E-3}{17}$...	5.54	
$\frac{E-3}{18}$...	5.64	
$\frac{E-3}{19}$...	5.54	
$\frac{E-3}{20}$...	5.74	17.02	19.33	
$\frac{E-3}{21}$...	5.39	18.12	20.45	

TABLE VI—*contd.*

No.	Nitrogen percentage.	Oil percentage.	Oil percentage on the dry substance.	REMARKS.
$\frac{E-3}{22}$...	5.80	
$\frac{E-3}{23}$...	5.41	
$\frac{E-3}{24}$...	5.68	
$\frac{E-3}{25}$...	5.38	17.30	19.84	
$\frac{E-3}{26}$...	5.57	16.90	19.33	
$\frac{E-3}{27}$...	5.58	17.54	19.66	
$\frac{E-3}{28}$...	5.92	17.56	19.77	
$\frac{E-3}{29}$...	5.56	17.46	19.65	
$\frac{E-3}{30}$...	5.82	18.06	20.29	
$\frac{E-3}{31}$...	5.76	17.26	19.18	
$\frac{E-3}{32}$...	5.63	16.74	19.05	
$\frac{E-3}{33}$...	6.14	17.78	20.10	
$\frac{E-3}{34}$...	5.74	17.68	19.92	
$\frac{E-3}{35}$...	5.64	15.76	17.92	
$\frac{E-3}{36}$...	5.75	
$\frac{E-3}{37}$...	5.75	15.26	17.54	

TABLE VI—*contd.*

No.		Nitrogen percentage.	Oil percentage.	Oil percentage on the dry substance.	REMARKS.
$\frac{E-3}{38}$...	5.83	
$\frac{E-3}{39}$...	5.64	14.68	16.77	
$\frac{E-3}{40}$...	5.37	
$\frac{E-3}{41}$...	5.78	16.90	19.06	
$\frac{E-3}{42}$...	5.60	
$\frac{E-3}{43}$...	5.98	16.84	18.95	
$\frac{E-3}{44}$...	5.66	16.60	18.76	
$\frac{E-3}{45}$...	5.42	16.56	18.74	
$\frac{E-3}{46}$...	5.27	
$\frac{E-3}{47}$...	5.87	
$\frac{E-3}{48}$...	5.85	15.22	17.24	
$\frac{E-3}{49}$...	5.89	
$\frac{E-3}{50}$	
$\frac{E-3}{51}$...	5.46	
$\frac{E-3}{52}$...	5.29	16.08	18.24	
$\frac{E-3}{53}$...	5.59	

TABLE VI—*contd.*

No.		Nitrogen percentage.	Oil percentage.	Oil percentage on the dry substance.	REMARKS.
$\frac{E-3}{54}$...	5.49	17.18	19.33	
$\frac{E-3}{55}$...	6.08	18.14	
$\frac{E-3}{56}$...	5.88	16.90	18.84	
$\frac{E-3}{57}$...	5.89	17.80	19.84	
$\frac{E-3}{58}$...	5.98	
$\frac{E-3}{59}$...	5.50	18.02	20.09	
$\frac{E-3}{60}$...	6.10	15.98	
$\frac{E-3}{61}$...	5.63	
$\frac{E-3}{62}$...	5.90	
$\frac{E-3}{63}$...	5.81	
$\frac{E-3}{64}$...	5.74	
$\frac{E-3}{65}$...	5.85	17.86	19.87	
$\frac{E-3}{66}$...	5.83	
$\frac{E-3}{67}$...	5.29	
$\frac{E-3}{68}$...	5.91	
$\frac{E-3}{69}$...	5.39	18.10	19.88	

TABLE VI--*contd.*

No.	Nitrogen percentage.	Oil percentage.	Oil percentage on the dry substance.	REMARKS.
$\frac{E-3}{70}$...	5.47	
$\frac{E-3}{71}$...	5.51	
$\frac{E-3}{72}$...	5.82	14.20	15.62	
$\frac{E-3}{73}$...	5.52	
$\frac{E-3}{74}$...	5.63	
$\frac{E-3}{75}$..	5.35	17.38	19.29	
$\frac{E-3}{76}$...	6.04	16.12	17.96	
$\frac{E-3}{77}$..	6.11	17.22	
$\frac{E-3}{78}$...	5.85	
$\frac{E-3}{79}$...	5.53	
$\frac{E-3}{80}$...	5.89	
$\frac{E-3}{81}$...	5.80	16.70	18.42	
$\frac{E-3}{82}$...	5.95	
$\frac{E-3}{83}$...	4.80	19.06	
$\frac{E-3}{84}$...	5.20	
$\frac{E-3}{85}$...	5.68	

TABLE VI—*contd.*

No.	Nitrogen percentage.	Oil percentage.	Oil percentage on the dry substance.	REMARKS.
$\frac{E-3}{86}$...	4.96	17.76	
$\frac{E-3}{87}$...	5.46	
$\frac{E-3}{88}$...	5.75	
$\frac{E-3}{89}$...	5.74	
$\frac{E-3}{90}$...	5.17	
$\frac{E-3}{91}$...	5.68	
$\frac{E-3}{92}$...	5.84	
$\frac{E-3}{93}$...	5.47	
$\frac{E-3}{94}$...	5.92	
$\frac{E-3}{95}$...	5.43	
$\frac{E-3}{96}$...	5.50	
$\frac{E-3}{97}$...	5.79	
$\frac{E-3}{98}$...	5.74	
$\frac{E-3}{99}$...	5.65	
$\frac{E-3}{100}$...	5.82	15.96	17.74	
$\frac{E-3}{101}$...	6.18	16.12	

TABLE VI—*contd.*

No.		Nitrogen percentage.	Oil percentage.	Oil percentage on the dry substance.	REMARKS.
E—3 102	...	5.45	
E—3 103	...	6.01	17.82	20.09	
E—3 104	...	5.50	16.90	19.03	
E—3 105	...	5.53	15.66	17.52	
E—3 106	...	5.64	
E—3 107	...	5.46	
E—3 108	...	6.04	16.14	18.97	
E—3 109	...	5.41	17.48	18.27	
E—3 110	...	5.65	
E—3 111	...	5.67	
E—3 112	...	5.62	17.46	19.02	
E—3 113	...	5.34	16.60	18.11	
E—3 114	...	5.91	16.78	18.27	
E—3 115	...	5.09	
E—3 116	...	5.89	
E—3 117	...	5.43	17.76	19.95	

TABLE VI—*contd.*

No.	Nitrogen percentage.	Oil percentage.	Oil percentage on the dry substance.	REMARKS.
$\frac{E-3}{118}$...	5.01	18.30	...	
$\frac{E-3}{119}$.	5.33	16.36	18.37	
$\frac{E-3}{120}$...	5.27	17.10	19.20	
$\frac{E-3}{121}$...	5.76	
$\frac{E-3}{122}$...	5.78	
$\frac{E-3}{123}$...	5.58	
$\frac{E-3}{124}$...	5.50	17.46	19.63	
$\frac{E-3}{125}$...	5.55	
$\frac{E-3}{126}$...	5.77	
$\frac{E-3}{127}$...	5.76	
$\frac{E-3}{128}$...	5.60	
$\frac{E-3}{129}$...	5.77	
$\frac{E-3}{130}$...	5.74	
$\frac{E-3}{131}$...	5.01	17.06	
$\frac{E-3}{132}$...	5.35	
$\frac{E-3}{133}$...	5.63	

TABLE VI—*concl.*

No.	Nitrogen percentage.	Oil percentage.	Oil percentage on the dry substance.	REMARKS.
$\frac{E-3}{134}$...	5.40	
$\frac{E-3}{135}$...	5.77	
$\frac{E-3}{136}$...	5.38	
$\frac{E-3}{137}$...	5.49	
$\frac{E-3}{138}$...	5.55	
$\frac{E-3}{139}$...	5.80	
$\frac{E-3}{140}$...	5.53	
$\frac{E-3}{141}$...	5.33	
$\frac{E-3}{142}$...	5.56	
$\frac{E-3}{143}$...	5.40	
$\frac{E-3}{144}$...	5.43	
$\frac{E-3}{145}$...	4.90	19.32	
$\frac{E-3}{146}$...	5.12	
$\frac{E-3}{147}$...	5.24	
$\frac{E-3}{148}$...	5.69	
$\frac{E-3}{149}$...	5.73	
$\frac{E-3}{150}$...	5.34	

TABLE VII.

Results obtained in 1911 with 152 samples of chocolate soy beans (Type 4).

No.	Nitrogen percentage.	Oil percentage.	Oil percentage on the dry substance.	REMARKS.
$\frac{E-2}{1}$...	4.93	17.62	19.55	The oil content in the dry substance was calculated, but was found to make no difference to our conclusions. In consequence the actual percentages observed in the undried substance have been used in the letter-press.
$\frac{E-2}{2}$...	5.66	17.67	19.88	
$\frac{E-2}{3}$...	4.75	18.22	20.00	
$\frac{E-2}{4}$...	5.57	16.46	18.62	
$\frac{E-2}{5}$...	4.73	19.16	20.75	
$\frac{E-2}{6}$...	5.54	16.42	18.64	
$\frac{E-2}{7}$...	5.35	16.00	18.13	
$\frac{E-2}{8}$...	5.12	16.51	16.65	
$\frac{E-2}{9}$...	7.08	
$\frac{E-2}{10}$...	Spoilt.	
$\frac{E-2}{11}$...	5.55	
$\frac{E-2}{12}$...	4.92	18.16	19.89	
$\frac{E-2}{13}$...	5.88	
$\frac{E-2}{14}$...	5.16	16.90	18.73	
$\frac{E-2}{15}$...	5.57	16.94	19.28	

TABLE VII—*contd.*

No.		Nitrogen percentage.	Oil percentage.	Oil percentage on the dry substance.	REMARKS.
$\frac{E-2}{16}$...	Spoilt.	16.34	18.61	
$\frac{E-2}{17}$...	5.24	
$\frac{E-2}{18}$...	6.00	15.60	17.61	
$\frac{E-2}{19}$...	5.68	16.40	18.52	
$\frac{E-2}{20}$...	5.36	17.08	19.12	
$\frac{E-2}{21}$...	5.65	
$\frac{E-2}{22}$...	5.44	17.26	19.39	
$\frac{E-2}{23}$...	5.44	16.56	18.68	
$\frac{E-2}{24}$...	5.49	17.62	19.95	
$\frac{E-2}{25}$...	5.77	16.58	18.78	
$\frac{E-2}{26}$...	5.68	17.40	19.64	
$\frac{E-2}{27}$...	6.10	16.70	18.86	
$\frac{E-2}{28}$...	5.57	15.20	17.7	
$\frac{E-2}{29}$...	Spoilt.	
$\frac{E-2}{30}$...	5.35	16.06	18.27	
$\frac{E-2}{31}$...	4.98	

TABLE VII—*contd.*

No.	Nitrogen percentage.	Oil percentage.	Oil percentage on the dry substance.	REMARKS.
$\frac{E-2}{32}$...	5.18	17.30	19.51	
$\frac{E-2}{33}$...	5.64	17.20	19.20	
$\frac{E-2}{34}$...	5.59	16.90	19.00	
$\frac{E-2}{35}$...	5.33	16.66	18.58	
$\frac{E-2}{36}$...	6.12	17.30	19.07	
$\frac{E-2}{37}$...	5.44	7.50	19.92	
$\frac{E-2}{38}$..	5.21	16.94	18.91	
$\frac{E-2}{39}$...	5.96	17.22	19.17	
$\frac{E-2}{40}$...	5.34	16.68	18.54	
$\frac{E-2}{41}$...	5.98	
$\frac{E-2}{42}$...	6.08	17.54	19.55	
$\frac{E-2}{43}$...	5.44	
$\frac{E-2}{44}$...	5.24	17.72	20.24	
$\frac{E-2}{45}$...	5.49	17.08	19.56	
$\frac{E-2}{46}$...	5.47	17.47	19.91	
$\frac{E-2}{47}$...	5.39	

TABLE VII --*contd.*

No.	Nitrogen percentage.	Oil percentage.	Oil percentage on the dry substance.	REMARKS
$\frac{E-2}{48}$	5.28	16.88	19.31	
$\frac{E-2}{49}$...	5.33	
$\frac{E-2}{50}$	5.24	17.08	19.31	
$\frac{E-2}{51}$	5.80	16.40	18.43	
$\frac{E-2}{52}$...	5.24	
$\frac{E-2}{53}$...	5.72	
$\frac{E-2}{54}$...	5.41	17.80	19.97	
$\frac{E-2}{55}$...	5.32	18.32	20.52	
$\frac{E-2}{56}$..	5.48	16.58	18.81	
$\frac{E-2}{57}$...	5.53	
$\frac{E-2}{58}$..	6.23	15.84	17.97	
$\frac{E-2}{59}$...	5.57	16.78	18.71	
$\frac{E-2}{60}$...	5.82	17.16	19.14	
$\frac{E-2}{61}$...	6.03	16.28	18.76	
$\frac{E-2}{62}$..	5.35	16.12	18.46	
$\frac{E-2}{63}$...	5.40	17.36	19.66	

TABLE VII—*contd.*

No.	Nitrogen percentage.	Oil percentage.	Oil percentage on the dry substance.	REMARKS.
$\frac{E-2}{64}$...	5.62	17.20	19.49	
$\frac{E-2}{65}$...	5.35	16.68	18.81	
$\frac{E-2}{66}$..	6.06	17.02	18.60	
$\frac{E-2}{67}$...	5.58	
$\frac{E-2}{68}$...	5.61	16.80	18.94	
$\frac{E-2}{69}$..	5.38	
$\frac{E-2}{70}$..	5.84	16.28	18.63	
$\frac{E-2}{71}$...	6.01	16.50	18.66	
$\frac{E-2}{72}$...	6.25	17.70	19.43	
$\frac{E-2}{73}$..	5.94	17.00	19.04	
$\frac{E-2}{74}$...	5.42	
$\frac{E-2}{75}$...	5.89	16.76	18.78	
$\frac{E-2}{76}$..	5.41	
$\frac{E-2}{77}$...	5.93	16.66	19.25	
$\frac{E-2}{78}$...	4.97	
$\frac{E-2}{79}$...	5.87	

TABLE VII—*contd.*

No.		Nitrogen percentage.	Oil percentage.	Oil percentage on the dry substance.	REMARKS.
$\frac{E-2}{80}$...	5.95	18.68	21.45	
$\frac{E-2}{81}$...	5.73	18.18	20.96	(Slight powder car- ried over.)
$\frac{E-2}{82}$...	4.83	19.30	21.11	Dry, analysed in hot weather.
$\frac{E-2}{83}$...	5.07	19.44	21.11	
$\frac{E-2}{84}$...	5.53	16.81	19.32	
$\frac{E-2}{85}$...	5.77	
$\frac{E-2}{86}$...	4.93	19.38	20.82	Dry, analysed in hot weather.
$\frac{E-2}{87}$...	5.33	
$\frac{E-2}{88}$...	5.35	17.20	19.61	
$\frac{E-2}{89}$...	5.30	
$\frac{E-2}{90}$...	5.16	18.06	20.66	
$\frac{E-2}{91}$...	5.89	17.86	20.41	
$\frac{E-2}{92}$...	5.56	17.12	19.89	
$\frac{E-2}{93}$...	5.80	16.80	19.46	
$\frac{E-2}{94}$...	5.66	17.38	19.73	
$\frac{E-2}{95}$...	5.89	

TABLE VII—*contd.*

No.	Nitrogen percentage.	Oil percentage,	Oil percentage on the dry substance.	REMARKS.
$\frac{E-2}{96}$..	6.04	15.72	17.86	
$\frac{E-2}{97}$...	5.81	16.98	19.23	
$\frac{E-2}{98}$..	5.93	
$\frac{E-2}{99}$..	6.01	
$\frac{E-2}{100}$...	5.21	
$\frac{E-2}{101}$...	Spoilt.	
$\frac{E-2}{102}$...	5.41	
$\frac{E-2}{103}$...	5.72	
$\frac{E-2}{104}$...	5.49	
$\frac{E-2}{105}$...	5.77	
$\frac{E-2}{106}$...	5.58	
$\frac{E-2}{107}$...	5.73	
$\frac{E-2}{108}$...	5.66	
$\frac{E-2}{109}$	
$\frac{E-2}{110}$	
$\frac{E-2}{111}$...	5.24	

TABLE VII---*contd.*

No.	Nitrogen percentage.	Oil percentage.	Oil percentage on the dry substance.	REMARKS.
$\frac{E-2}{112}$...	5.89	
$\frac{E-2}{113}$...	5.96	
$\frac{E-2}{114}$...	5.45	
$\frac{E-2}{115}$...	6.06	
$\frac{E-2}{116}$...	5.55	
$\frac{E-2}{117}$...	5.76	
$\frac{E-2}{118}$...	5.64	
$\frac{E-2}{119}$...	5.10	
$\frac{E-2}{120}$...	5.53	
$\frac{E-2}{121}$	
$\frac{E-2}{122}$...	5.69	
$\frac{E-2}{123}$...	5.57	
$\frac{E-2}{124}$...	5.88	
$\frac{E-2}{125}$...	Spoilt.	
$\frac{E-2}{126}$...	5.75	
$\frac{E-2}{127}$...	6.00	

TABLE VII—*contd.*

No.	Nitrogen percentage.	Oil percentage.	Oil percentage on the dry substance.	REMARKS.
$\frac{E-2}{128}$..	5.88	
$\frac{E-2}{129}$...	5.19	
$\frac{E-2}{130}$...	5.12	
$\frac{E-2}{131}$...	5.42	
$\frac{E-2}{132}$...	4.86	
$\frac{E-2}{133}$...	5.83	
$\frac{E-2}{134}$...	5.98	
$\frac{E-2}{135}$...	5.67	
$\frac{E-2}{136}$...	5.87	
$\frac{E-2}{137}$...	5.65	
$\frac{E-2}{138}$...	5.36	
$\frac{E-2}{139}$...	5.37	
$\frac{E-2}{140}$...	5.26	
$\frac{E-2}{141}$...	5.31	
$\frac{E-2}{142}$...	5.59	
$\frac{E-2}{143}$...	5.20	

TABLE VII--*concl.*

No.		Nitrogen percentage.	Oil percentage.	Oil percentage on the dry substance.	REMARKS.
$\frac{E-2}{144}$...	5.09	
$\frac{E-2}{145}$...	5.58	
$\frac{E-2}{146}$...	5.35	
$\frac{E-2}{147}$...	5.66	
$\frac{E-2}{148}$...	5.85	
$\frac{E-2}{149}$...	5.46	
$\frac{E-2}{150}$...	5.86	
$\frac{E-2}{151}$...	5.90	
$\frac{E-2}{152}$...	5.96	

TABLE VIII.

Life periods of Bengal Varieties of Soy Beans grown in Bengal.

Variety.	Where grown.	Date of planting.	Date of harvesting.	Life period.	
Type 1. Late black seed.	Sabour	June 3, 1910	Dec. 20, 1910...	200 days	
	"	" 9, 1911	Dec. 19, 1911...	193 "	
	"	July 12, 1911	Dec. 15, 1911...	156 "	
	Bankipur	June 1, 1911	Nov. 24 to 30, 1911.	177—183 days	
	Dumraon	July 10, 1911	Dec. 28, 1911...	171 days	
	Outtack	June 12, 1911	Jan. 1912...	210 "	
	Chinsura	" 10, 1911	Dec. 25, 1911...	199 "	on clay loam.
	"	July 1, 1911	Dec. 28, 1911...	181 "	on loam.
Type 1 A.	Kalimping	June 15 to July 7, 1911	Nov. 16 to Dec. 2, 1911.	132—170 days	
Type 2. Early black seed.	Sabour	July 12, 1911	Nov. 26, 1911...	137 days	
Type 3. Yellow seed.	"	June 3, 1910	Dec. 22, 1910...	202 "	
	"	" 9, 1911	" 19, 1911...	193 "	
	"	July 12, 1911	" 20, 1911...	161 "	
	Chinsura	June 10, 1911	" 25, 1911...	199 "	
Type 3 A. Yellow seed.	"	July 1, 1911	" 28, 1911...	181 "	
	Sabour	" 12, 1911	Nov. 18 to Dec. 15, 1911.	129—156 days	
	Kalimping	June 15 to July 7, 1911	Nov. 16 to Dec. 2, 1911.	132—170 days	
Type 4. Chocolate seed.	Sabour	June 3, 1910	Dec. 22, 1910...	202 days.	
	"	June 9, 1911	Dec. 19, 1911...	193 "	
	"	July 12, 1911	Dec. 20, 1911...	161 "	
	Bankipur	June 1, 1911	Nov. 24 to 30, 1911.	177—183 days.	
	Dumraon	July 10, 1911	Dec. 26, 1911...	169 days.	
Type 4 A. Pale chocolate.	Outtack	June 12, 1911	Jan. 7, 1912 ..	210 "	
	Sabour	July 12, 1911	Nov. 18 to Dec. 13, 1911.	129—154 days.	
	Kalimping	June 15 to July 7, 1911.	Nov. 16 to Dec. 2, 1911.	132—170 "	
Type 5. Barmeli	Sabour	July 12, 1911	Nov. 18, 1911...	129 days.	
	Kalimping	June 27, 1910	" 15, 1910 ..	141 "	
	"	June 15 to July 7, 1911.	" 16 to Dec. 2, 1911.	132—170 days.	
Type 6. Nepali	Sabour	July 12, 1911	Oct. 30, 1911 ..	111 days.	
	Kalimping	June 2, 1910	" 6, 1910 ..	126 "	
	"	May 10, 1911	" 16, 1911 ..	159 "	



LIST OF PLATES.

Plate I ...	Type I ..	Late Black seeded variety—photographed, October 28, 1911.
Plate II ...	Type V ..	Barmeli variety—photographed, October 28, 1911. <i>N. B.</i> —Some leaves have been removed to show the number of pods formed.
Plate III ...	Type VI ...	Nepali variety on left.
	Type IIIa ...	Upright long branched greeny yellow seeded variety on right.
Plate IV ...		Barchot variety—photographed, October 30, 1911. <i>N. B.</i> —The erect scale in each plate measures one metre.

PLATE I.

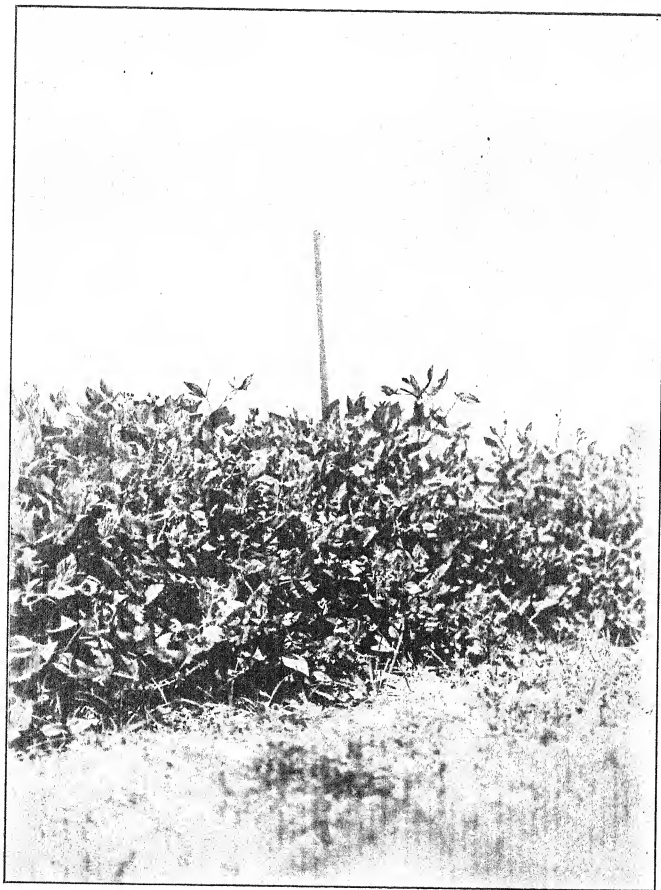
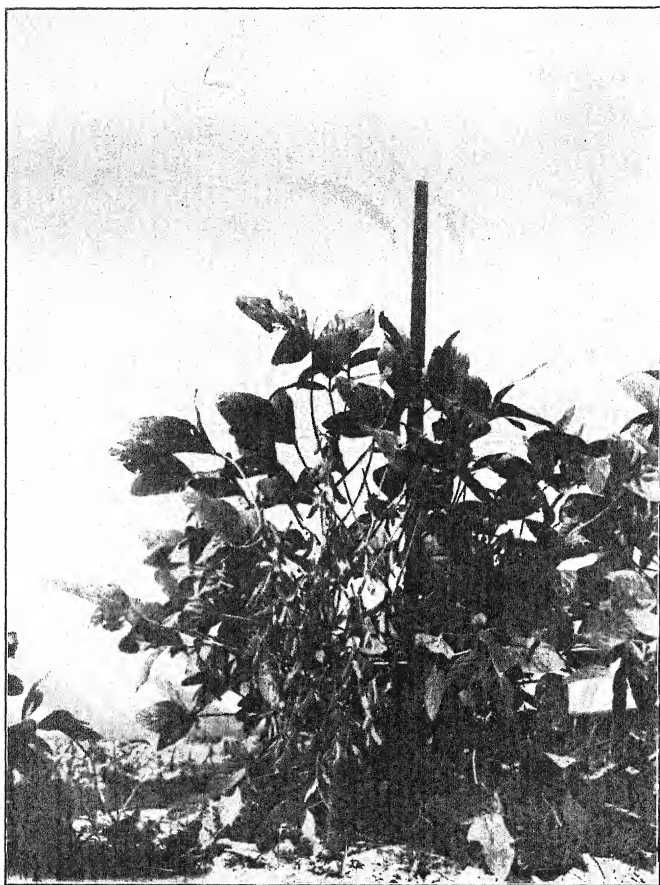




PLATE II.



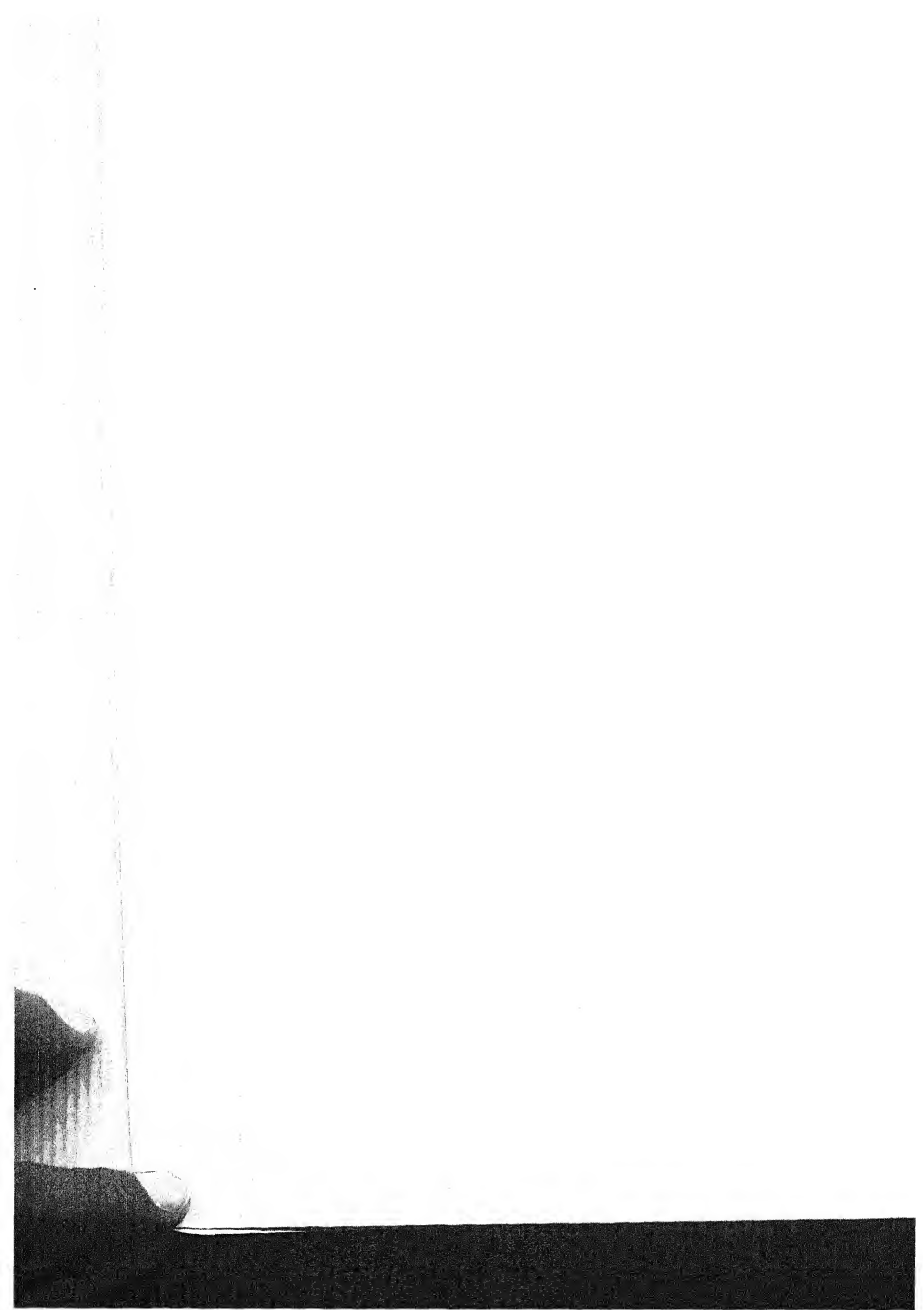
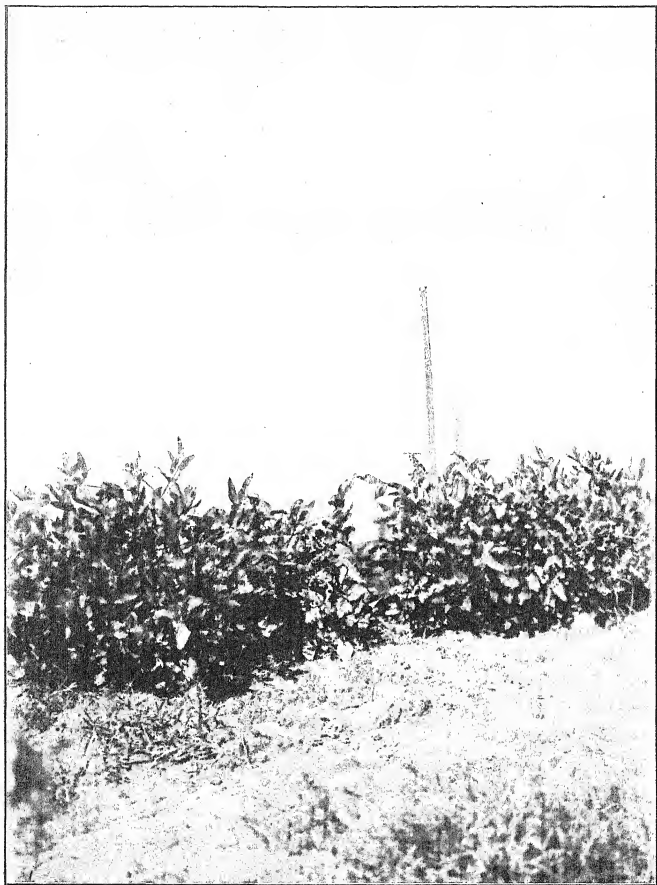


PLATE III.



PLATE IV.



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ON PHYTOPHTHORA PARASITICA NOV. SPEC.

A NEW DISEASE OF THE CASTOR OIL PLANT.

BY

JEHANGIR FARDUNJI DASTUR, B.Sc.,

First Assistant to the Imperial Mycologist.



AGRICULTURAL RESEARCH INSTITUTE, PUSA

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FOREWORD.

The study of *Phytophthora* on *Ricinus* was undertaken at the suggestion of Dr. E. J. Butler, M.B., F.L.S., the Imperial Mycologist. I have to express my gratitude to him for his ever ready help and invaluable advice which I have received from him. I have also to express my thanks to Mr. F. J. F. Shaw, B.Sc., A.R.C.S., F.L.S., the Supernumerary Mycologist, for the microphotographs taken by him.

J. F. D.



ON PHYTOPHTHORA PARASITICA NOV. SPEC.
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INTRODUCTION.

THE castor oil plant (*Ricinus communis*, L.), a native of India, according to some botanists and according to others a native of Abyssinia and Tropical Africa, is largely cultivated on account of its multifarious uses not only in India, but also in South United States, Southern Africa, Tropical Africa, Algeria, Sicily, Southern France, Brazil, Java, West Indies and other tropical and subtropical regions. In India and elsewhere the castor oil plant is generally grown as a field-border and as a mixed crop but rarely as a sole crop. The plant is extensively cultivated in Surat, Ahmedabad, Dharwar and Belgaum in the Bombay Presidency. In the United Provinces castor is more or less grown everywhere, especially in Allahabad, where it occupies as a sole crop about 1,200 to 1,300 acres, chiefly along the banks of the Jumna. In the Madras Presidency it is widely in cultivation, especially in Salem and Bellary. In Bengal not much castor is to be found. In Assam it is never cultivated as a chief crop but in almost every ryot's garden small areas are allotted to it. The products of the castor oil plant, on account of their varied uses in industry, agriculture and the arts, are of great economic importance. The oil obtained from its seed, the castor oil of commerce, is in great demand. Though a large part of India's produce is locally consumed, still in the year 1910-11 India exported 1,203,007 gallons of the oil, worth Rs. 1,690,455 and 2,148,033 cwts.

of seed worth Rs. 16,499,640. The oil is greatly used in the manufacture of the famous Turkey-red. Again its medical properties secure for it an extensive trade. In many parts of India it is used as a luminant as it gives a bright smokeless light. Especially in Australia and some other places it finds a wide use as a lubricant. It is also employed for dressing tanned hides and skins. The castor oil-cake is an important constituent of manures. In Assam the plant is cultivated for feeding the *eri*-silk worm on its leaves. The growing cottage *eri*-silk industry will necessitate its wider cultivation for this purpose.

Though the castor oil plant is so widely distributed still no important fungus pest, except the rust,¹ has been recorded as attacking this crop; it has been considered to be comparatively free from fungus enemies, and yet two serious pests occur at Pusa. One is *Phytophthora parasitica* nov. spec., the other a species of *Cercospora*. The latter is confined only to leaves and so does not prove fatal to the plant. In wet weather this disease is a serious menace to the cultivation of this crop for the *eri*-silk industry, as about ninety per cent. of the leaves of old and young plants are sometimes covered all over with *Cercospora* spots. To a greater or less extent this disease is present all the year round.

In the wet months of August and September *Choanephora* attacks leaves, growing points and tender shoots. This parasite sets up a rot in the affected parts.

There is also an *Alternaria* found occurring on the leaves which might be parasitic.

All these diseases will be dealt with in another paper when their study is completed.

Phytophthora on castor was first found at Pusa in the year 1909 in August. Since then it has been found appearing every year about the end of June, after the rains have set in. Last year (1912) it appeared as early as June on account of the frequent showers of

¹ Ajrekar, S. L. The Castor rust (? *Melampsorella Ricini*, de Toni). Jour. Bombay Nat. Hist. Soc. 1912, p. 1092.

rain we had in April and May. The disease disappears by the beginning of September, at the end of the rains. About nineteen varieties were grown on the Pusa Farm in 1911, but none of them escaped the attack of this fungus, not even the indigenous Patna variety.

In no paper dealing with castor cultivation except in one has any reference been made concerning the high mortality of the seedlings or concerning the characteristic brown leaf spots found on leaves of young and old plants.

Darrah¹ reports that in the year 1889 "a very large number of young castor plants, even those from the indigenous Cachar seed, had damped off" on account of damp and excessive rain. Mr. Mackenzie, who was in charge of the *eri*-silk experiments, observed that the seedlings damped off and died "before they matured a sufficiently hard skin or bark on their stems."

This damping off of seedlings in water-logged places is what I have found on the Pusa Farm to be due to *Phytophthora parasitica*. In all probability therefore *Phytophthora* was the cause of the large destruction of castor seedlings of Mr. Mackenzie's crop in 1889.

This parasite is the most injurious of the fungal parasites of castor; it destroys seedlings and attacks leaves of older plants. My thanks are due to Mr. M. N. De, the Sericulture Assistant at Pusa, for carrying out experiments to see the effect of feeding *eri*-silk worms on leaves attacked by *Phytophthora*. He informs me that the worms refused to eat the diseased portions of the leaves, and that their growth when fed on diseased leaves was very slow as compared with those fed on healthy leaves.

In the case of a monsoon (Kharif) crop the most critical period in its life history is when the seedlings are about six to eight inches high and when their stems are quite tender. In low-lying water-logged and badly drained fields about thirty to forty per cent. of the seedlings damp off. The first indication of the disease is the appearance of a roundish patch of an unhealthy dull green colour on both the surfaces of a cotyledon. It soon helplessly

¹ Darrah, H. Z. *The Eri-silk of Assam*, 1890, p. 15.



hangs down from its point of attachment. The disease spreads from the leaf to the petiole, thence to the stem and the growing point, killing the seedling. In older plants the disease in the field is localised on the lamina or leaf-blade. As the diseased spot gets older it turns yellowish and then brown. It spreads concentrically, and in old spots forms concentric brown rings enclosing lighter brown areas (Plate I). They are surrounded on the upper surface by a yellowish green or dull pale green ill-defined border. On the under surface the old spots have a brownish grey and ill-defined border which is relieved occasionally with very minute white specks. The brown central portion of the spots is always very sharply contoured. The diseased areas either remain isolated or become confluent, often covering almost the whole of the leaf. In case of confluent spots the successive periods of their growth are not marked by concentric rings but by wavy margins of different shades of brown. The spots are not limited by the main ribs, even in the case of big mature leaves. The diseased leaves have a tendency to fall off prematurely. Growing points and leaf-buds of plants of all ages are protected from the external invasion of the disease by the waxy sheathing stipules which completely cover them. Petioles of big leaves, stems with thick epidermis, flowers and fruits, have been found till now in nature to be immune from the ravages of the fungus.

On the under surface of the leaf is seen with the aid of a pocket lens a sparse white web of threads originating from the unhealthy green coloured portion of the diseased spot. These are the long and unbranched sporangiophores, measuring from 35 to over 500 μ , as a rule 100 to 300 μ . They emerge from within the leaf as a rule through stomata or between the cell walls of two contiguous cells (Plate II, Fig. 1). They have been occasionally found to make their way out by breaking through the upper wall of the epidermis. They make their exit from the leaf singly, rarely in twos or threes but never in clusters.

In the petiole and stems it is a purely physical matter for the hypha to make its way out of the tissues. Just underneath the upper wall of the epidermis the hypha swells into a globular head and

under its pressure the wall gives way (Plate II, Fig. 6). In petioles and in stems the hypha emerges singly as a rule, as in the case of leaves, but occasionally a cluster of four or five have been found to come out from the same point (Plate II, Fig. 7).

The mycelium within the tissues is both intracellular and intercellular. Wherever an intracellular hypha passes through a wall there is invariably a constriction of the hypha (Plate II, Fig. 2). Haustoria are very scarce. Hartig¹ has described them to be button-shaped in *Ph. Fagi* (*Ph. omnivora* de Bary), Coleman² finger-shaped in *Ph. omnivora*, var. *Areceæ*, Klebahn³ short thick and finger-like in *Ph. Syringæ* and *Ph. omnivora*, while de Bary says, "Bestimmte Haustorien sind nicht vorhanden" but in *Ph. parasitica* both finger-shaped and button-shaped haustoria have been found (Plate III, Figs. 2 B and 3); they branch very rarely. Whether these bodies which we call haustoria are true haustoria or the initial stage of branches of the intracellular hyphæ arising from the intercellular mycelium remains an open question. No cellulose cap has been observed at the tips of these finger-like or button-shaped bodies which would leave no doubt as to their identity. In Plate III, Fig. 2, A is distinctly a branch of the intercellular hypha but B may be a haustorium or a branch like A in a very early stage; but, on the other hand, in Plate III, Fig. 4, A appears to be a branched haustorium.

When a diseased petiole or stem is cut transversely, the ring of fibro-vascular bundles becomes prominent by its black or brown colour on account of the fungus choking the vessels (Plate II, Fig. 2). *Ph. omnivora* de Bary and *Ph. omnivora*, var. *Areceæ* Coleman have been known to attack the vascular bundles. The hyphæ of *Ph. parasitica* enter and leave the vessels of the fibrovascular bundles through their unthickened portions. The hypha at the point of entrance or exit generally swells, the thin

¹ Hartig, R. Untersuchungen aus dem forstbotanischen Institut zu München, 1880, p. 41.

² Coleman, L. C. Diseases of the Areca Palm. Dept. of Agric., Mysore State, Mys. Ser. Bull. No. 2, 1910, p. 82.

³ Klebahn, H. Krankheiten des Flieders, 1909, pp. 42 and 43.

membranous portion gives way, possibly under pressure and the fungus continues its course inwards or outwards as the case may be (Plate II, Fig. 3).

Internal sporangia have often been observed in the tissues of leaves, stems and fruits. They have not been found either to germinate conidially or to produce zoospores in leaves and stems, but in a few cases they have been observed to germinate conidially in the soft tissues of the pericarp of the fruit (Plate V, Fig. 15). Of what use they are to the parasite it is difficult to say. They are known to be formed in the leaf within twenty-four hours after inoculation. Similar bodies have been found in the leaves of potatoes attacked with *Ph. infestans*.

I have failed up to now to find typical oospores in nature. It is possible that they are formed, as in some artificial media they are readily produced, but they may be very few and so escape detection. Very rarely on mashing old leaves boiled in caustic potash resting bodies have been found which might be parthogenetic oospores.

INFECTION EXPERIMENTS ON THE CASTOR OIL PLANT.

From the many series of inoculation experiments carried out in the laboratory it has been found that leaves young and old most readily fall victims to the attack of the parasite. Before it was taken in pure culture inoculations were made in either of the two following ways:—(1) A bit from a diseased portion of a leaf from the field was placed with a drop of water on either of the surfaces of a leaf of a healthy plant which was then covered by a bell jar to keep it in a moist atmosphere. After about six hours the diseased piece used for inoculation was removed. (2) A diseased leaf from the field was kept for twenty-four hours slightly immersed in water in a covered Petri dish. The fungus grew out on the surface of the water in the Petri dish and produced sporangia in abundance; from the surface not in contact with water an aerial growth was produced. Either zoospores suspended in water or a little of the mycelium

¹ Jones, L. R., Giddings, N. J., and Lutman, B. F. Investigations of the Potato Fungus *Phytophthora infestans*. U.S. Dept. Agric., Bureau of Plant Industry, Bull. No. 245, 1912, p. 28.

were used for inoculating healthy leaves. When the fungus was taken in culture, zoospores suspended either in distilled water or in sterilised tap water were invariably used for the various inoculations. Whatever was the technique employed for the inoculation the result was always the same. The effect of the inoculation was clearly perceptible within twenty-four hours by the inoculated area taking a dull unhealthy green colour. Sporangia were produced in about forty-eight hours after inoculation, when the inoculated leaf was kept moist by occasionally spraying it with water. The inoculated leaves could be easily induced to produce sporangia by suspending them in tap water. They readily discharged their zoospores when transferred to a drop of fresh water. These zoospores were used to inoculate a new set of leaves of healthy plants. The inoculation took as readily as in the previous experiments. In the case of leaves of tender seedlings and of very young leaves of bigger plants the disease spread from the lamina to the petiole, thence to the stem.

From these infection experiments it is found that the germ-tubes of the zoospores penetrate the leaf three to five hours after they are sown on it. The penetration takes place on either of the surfaces and is not accomplished necessarily through a stoma. They may even break through the upper wall of the epidermal cells or penetrate between the cell walls of two neighbouring cells (Plate III, Figs. 5-11). What factor stimulates the germ-tube to enter the host plant in one way or the other is difficult to say ; very often it has been found to cross over a stoma without entering it (Plate III, Fig. 10). Before the germ-tube enters the leaf its end is slightly enlarged as a rule.

We have seen that in nature the fungus is confined to seedlings and to leaves of older plants. Stems of tender seedlings about six to eight inches high are found diseased in nature. When a stem of a healthy tender seedling is inoculated by means of zoospores suspended in a drop of water, the inoculated area soon turns brown. The disease extends in both directions, ultimately reaches the

growing point and thereby kills the seedling. If the seedling is kept in a very moist atmosphere the inoculated stem produces in forty-eight hours a white fluffy aerial mycelial growth, bearing sporangia. The hyphæ project about a millimetre or two from the stem. But stems of plants out of the seedling stage seem to be immune from the attack and artificial inoculations have proved unsuccessful on these. When the ordinary method of inoculating, by means of putting a drop of water containing freshly discharged zoospores on these stems failed, Klebahn's¹ method, employed by him in inoculating leaf buds of lilac plants, was tried. The stems of young plants about three to six months old were surrounded by a jacket of distilled water, containing motile zoospores, in a glass tube about four inches long and three-fourths of an inch broad, the lower end of which was plugged by a rubber cork cut into two equal halves and having a central bore just big enough to enable the two halves to surround the stem without injuring it. This end was made water-tight by means of moulding wax (Plasticine) or paraffin; the upper end was kept open. A young seedling was inoculated in the same way. The result was that the stem and the base of the petioles of the seedling in two days distinctly showed marks of successful inoculation and in four days it wilted. The photo (Plate X), taken on the fourth day after inoculation, shows the progress of the attack from the petiole to the leaves. The plants three to six months old remained quite healthy. Only one plant out of half a dozen such inoculations on plants with matured bark, showed a small blackish patch about a couple of centimetres in length, in two days after inoculation. As the diseased area was found not to grow further, even after a week, sections were made at this place. A few hyphæ were found confined to the epidermal cells only. They were unable to grow further into the stem and the consequent lack of food killed them. The inoculation failed even when the stem was wounded. The reason of the parasite not attacking stems of plants as soon as they are out of the seedling stage is

¹ Klebahn, H. *loc. cit.*, p. 55.

not far to seek. The tender stem of a seedling has an uncuticularised epidermis, the outer wall of which readily permits the hyphæ to penetrate it, the cortex is soft and juicy and therefore easily succumbs to the ravages of the parasite; its growth is not entirely checked on reaching the vascular bundles which, as we have seen, are attacked through the unthickened portions of their vessels. On the other hand, the stem of an older plant presents obstacles in its way from the very beginning. The outer wall of the epidermis is highly cuticularised and so wards off an attack of the hyphæ and even if they get into the stem through stomata, the germinating tube cannot go further on account of the thick-walled and closely packed collenchymatous cells of the cortex. Apart from these reasons of physico-chemical importance there may be very possibly others of physiological importance. Colour is lent to this by the disease confining itself to the lamina of a mature leaf of young plants and not spreading to the petiole and thence to the stem. I have succeeded up to now in inoculating petioles of only very young leaves. The inoculation was made by placing distilled water containing motile zoospores in a cup of moulding wax (Plasticine) which surrounded a part of the petiole at the node and parts of the internodes above and below it. The stem did not take the inoculation but the petiole did, in two days. The stem was then attacked through the latter. The hyphæ which entered from the petiole extended up the stem and killed the growing point. The growth of fungus in the opposite direction was very slow. In these various experiments it was found that not only under field conditions is the leaf bud safe from the attack of the parasite, but also when inoculated artificially, on account of the sheathing stipules.

Infection experiments have also been carried out on flowers and fruits. For these experiments as well the inoculation material was zoospores from pure cultures, suspended in either distilled or tap water. Stalks bearing flowers and fruits were stood in water in flower vases; drops of water containing motile zoospores were placed on the flowers and fruits. After the inoculation the flower vases were kept in moist chambers. Stalks of flower and fruits kept as "checks"

were placed in exactly similar conditions. Male flowers took the inoculation in a day, turning brown and then black. The branched stamens were cobwebbed by hyphae, rich in sporangia. Green fruits in two days turned black and became soft at the point of inoculation. The disease then spread over the whole fruit and caused it to rot. Sporangia and "resting" conidia were abundantly produced. The attack was not confined only to the outer soft portion of the pericarp, but the hard woody portion also was found to contain stray strands of hyphae which made their way through the lignified cells by means of a very fine projection which bored through the hard cell wall (Plate II, Fig. 4). A fine web of mycelium was found in the cocci between the seed and the endocarp. The fungus extended there through the lignified cells or, as often happened, through the dissepiments of the loculi. The fleshy outgrowth on the seed, the caruncle, was then attacked by the hyphae and through it the immature seed. The hyphae completely filled the caruncle. They invariably traversed the cells through the pits in their thick cellulose walls. The course of the hyphae in the seed coat was difficult to trace. Inoculations on dried fruits invariably failed.

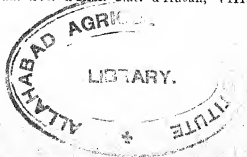
Though in the laboratory fruits and flowers are so virulently attacked by the parasite, still in nature they have been found to be entirely immune from its attack. The cause for this is solely to be attributed to the climatic conditions prevalent at the time of fruiting. The rainy (Kharif) crop fruits in October, November and December, a period when the fungus has completely disappeared from the fields. The winter (Rabi) crop fruits in April and May, when the temperature is too high for the fungus to grow.

The following experiments were undertaken to see how far soil inoculations prove successful.

On account of the frequent showers of rain in April and May last year (1912) the disease broke out early in June in self-sown plants in the castor plots of the previous year. About fifty to sixty per cent. of the seedlings were diseased. All the seedlings were removed from the field early in July. On the 17th three pots (Lot A) were filled

with earth from this diseased area. Care was taken to have the topmost layer of the earth in the field uppermost in the pots as well. Three seeds which were just germinating were removed to three pots with a big lump of earth (Lot B). Ten seeds were sown in each of the three pots of Lot A. On the 21st they germinated. On the 22nd one seedling of Lot B was attacked with *Phytophthora*. On the 23rd morning one of the seedlings of Lot A was suddenly found to have given way at the collar, at which place there was found an elliptical pale grey patch surrounded by a black brown border which in turn was enclosed by a light brown and not sharply defined ring. Sporangiophores were produced in a large quantity, breaking out through the epidermis at any point. On making sections internal sporangia were also found as far in as the pith. The cells of the pith and cortex were traversed by palmately branched or fasciculated hyphæ, the like of which have till now been observed only in some of the artificial media; wherever these hyphæ were found, the cell walls between two contiguous cells were partly or wholly dissolved (Plate II, Fig. 5). These palmately branched hyphæ have also been observed by Mangin¹ in *Plasmopora viticola*, *Pl. ribi*, *Pl. nivea* and some other *Peronosporaceæ* and according to him by MM. Milladet, Prillieux and Cavara as well, but these hyphæ, Mangin has found only in tissues surrounded by fibro-vascular bundles. He says, "cependant les tissus compacts qui entourent les nervures et qui cantonnent le mycélium dans des régions limitées, ne constituent pas une barrière infranchissable. En ces points, le mycélium s'aplatit et forme des lames ou des palmettes très élégamment ramifiées, qui s'insinuent dans les méats les plus étroits et parviennent même, soit mécaniquement, soit en digérant la substance intercellulaire, à dissocier un peu les cellules qui leur opposent de la résistance. La moindre fissure, la moindre fente, suffisent pour que le mycélium franchisse l'obstacle et pénètre dans une région saine; là, il évolue rapidement en pleine liberté au moyen des éléments nutritifs qu'il

¹ Mangin, L. Recherches sur les Péronosporées. Bull. Soc. d'Hist. Nat. d'Autun, VIII, 1895, p. 72.



trouve en abondance." I have found these palmately branched hyphæ in the cortical parenchymatous cells and growing away from the fibro-vascular bundles. The leaves and the growing point were not attacked when the seedlings gave way at the collar on being diseased. On the 24th another seedling from Lot B was diseased. The third remained healthy for a week more and then this part of the experiment ended. The seedlings from Lot A got attacked at the surface level of the soil one by one, till the majority of them died. The attack was never found to come from the roots upwards. It invariably began at the surface level of the earth in the pots.

All the pots of the Lots A and B, with some of the diseased seedlings were then kept in a moist condition in the laboratory. On the 30th of August one pot from Lot A was again sown with ten castor seeds. By the 5th of September eight seedlings died of the attack on the surface level. One of the two remaining seedlings was slightly attacked at the same place, but it succeeded in the struggle for existence. The other was not at all attacked.

It is needless to say that for all these experiments "checks" were kept in exactly the same condition, except that the earth was not from diseased areas.

On the 30th of September a second pot of Lot A was sown with castor seeds, *i.e.*, two months after the seedlings in this pot died of the attack. The seeds germinated after a week, and the seedlings grew into big plants without any being in the least diseased. This shows that the fungus in the soil was not able to retain its vitality for two months.

These experiments have proved interesting first because seedlings have been found to be attacked at the surface level of the earth got from diseased areas, while in the fields the attack has not been found to be at the surface level; secondly, because of the presence in the diseased tissues of the seedlings of palmately branched hyphæ, such as have been found growing in some culture media, while in diseased leaves and stems obtained from

the fields or by inoculations in the laboratory such hyphæ have never been seen.

PURE CULTURE.

Klebahn¹ succeeded in getting a pure culture of *Ph. omnivora* by inoculating agar in a Petri dish, directly from an infected beech seedling, but the *Phytophthora* on *Ricinus* could not be taken into pure culture by this method or by the ordinary poured plate method successfully employed by Rorer² for getting a pure culture of *Cacao Phytophthora*, possibly because this fungus gives only a submerged growth on glucose meat-extract agar, as was found when working with pure cultures. *Ph. infestans*³, *Phaseoli*⁴ *omnivora*, var. *Arecae*⁵ and *Colocasiae*⁶ have been taken into culture by cutting aseptic slabs from the diseased tubers, fruits and rhizomes ; a pure culture of *Ph. Syringæ* was got by Klebahn⁷ by incubating the rind of a diseased stem of *Syringa* ; evidently these methods could not be employed in the present case as in nature the fungus is found only on stems of tender seedlings six to eight inches high and on leaves. Flaming killed both the host and the parasite. Diseased leaves were suspended over sterilised pieces of carrot, potato, and in moist chambers on agar plates so as to allow conidia to drop on to the slabs and agar plates ; this method has been used with success by Rolfs⁸ in getting pure cultures of *Rhizoctonia* from the basidiospores of its *Corticium* stage ; with *Ph. parasitica* the media always got contaminated with bacteria and other saprophytes. Raciborski⁹ says he has grown *Ph. Nicotiana* in artificial culture but does not describe his technique.

¹ Klebahn, H. *loc. cit.* p. 70.

² Rorer, J. B. Pod-Rot, Canker and Chupon-Wilt of Cacao caused by *Phytophthora* Sp., Bull. of Trinidad Dept. of Agr., IX, No. 65, 1910, p. 14.

³ Clinton, G. P. Downy mildew or blight (*P. infestans* (Mont) de Bary), of Potato, II, 29th Report of the Connecticut Exp. Sta. 1905, p. 278.

⁴ *Ibid.*, Downy mildew or blight (*P. Phaseoli* Thaxt.) of Lima beans, *ib.*, p. 278.

⁵ Coleman, L. C. *loc. cit.*, p. 69.

⁶ At Pusa. An account of this species by Dr. E. J. Butler and Mr. G. S. Kulkarni is in the press.

⁷ Klebahn, H. *loc. cit.*, p. 37.

⁸ Rolfs, F. M. Potato Failures, Bull. 91, Colorado Expt. Sta. 1904, p. 10.

⁹ Raciborski, M. Parasit. Alg. Pilze Javas, Theil III, 1900, p. 5.

Trow¹, when working with *Pythium ultimum*, found it only necessary, in order to get a pure and copious growth of the fungus, to sterilise potatoes in suitable glass pots and inoculate them with a trace of the mycelium from the aerial growth developed on inoculated cabbage and rhubarb leaves projecting above the surface of water in Petri dishes. It, therefore, at first, seemed easy to get similarly a pure culture of *Ph. parasitica* from the aerial mycelial growth obtained about half a centimetre high in 24 to 48 hours by keeping a freshly diseased leaf in a Petri dish containing a little tap water, but when inoculations were made on sterilised potatoes, carrots, bread paste, acidified bread paste, glucose meat-extract agar, acidified glucose meat-extract agar, prune juice agar and *Ricinus* leaf juice agar, it was invariably found that bacteria contaminated the media and overgrew the fungus. Attempts were made to get a bit of a hypha free from bacteria by nipping it off just below its apex by means of a hot platinum needle but the hypha being unseptate the bit on the needle lost its protoplasm from its cut end; thus while getting rid of bacteria its vitality was destroyed. A living potato washed with formalin and cut into two by a sterile knife was inoculated on its cut surface. An aerial mycelial growth soon developed but again was not free from bacteria.

A pure culture was ultimately got by means of the following technique.

A diseased leaf was got from the field. It was well washed with sterilised water to get rid of foreign matter as much as possible and kept in a covered sterilised Petri dish containing a little sterilised tap water. The *Phytophthora* hyphæ spread out on the surface of the water and bore a crop of sporangia the next day. They readily discharged their zoospores in a drop of sterilised tap water. This drop was used to inoculate a clean well washed newly opened leaf of a seedling grown in the laboratory, thereby further minimising the presence of saprophytic fungi. The seedling was kept in a moist

¹ Trow, A. H. Observations on the Biology and Cytology of *Pythium ultimum*, n. sp. Ann. Bot., XV, 1901, p. 279.

atmosphere by covering it with a bell jar. The next day the inoculated leaf was plucked and immersed in 2.5 per cent. formalin for half an hour, it was then well washed with sterilised water and transferred to a sterilised covered Petri dish containing a little sterilised tap water. From the inoculated spot an aerial mycelial growth of *Phytophthora*, free from other fungi but not from bacteria, developed in 24 to 48 hours. It was found essential to keep the leaf in contact with water to get the aerial growth. A portion of the aerial mycelium was then transferred by means of a sterilised platinum needle to a piece of pith soaked in *Ricinus* leaf juice prepared as follows: About 10 to 15 grammes of green castor leaves were boiled for an hour in 100 c.c. of distilled water. One or two pieces of pith in an Erlenmeyer flask were soaked in some of the filtered decoction, enough to leave very little of it at the bottom of the flask after sterilisation. After two or three days a sparse woolly growth projected about a centimetre high from the upper surface of the pith. These hyphæ were free from bacteria and bore no sporangia except occasionally in old cultures, but when a bit was transferred to water, it bore innumerable sporangia the next day. If the flask contained much liquid the pith was soon overgrown with bacteria and the growth of the fungus retarded. It was now only necessary to inoculate a suitable culture medium with a bit of the mycelium growing on the pith. Such a medium was found in French bean (*Phaseolus vulgaris*) juice agar which gave in two days a pure, rich, healthy culture.

MORPHOLOGY OF THE FUNGUS IN CULTURE.

The comparatively young mycelium consists of thin, not much branched, unseptate hyphæ. The protoplasm within them is finely granular; but as they grow older they become very broad, up to 9μ ; the granules become coarser and aggregate into groups or irregular masses, the contents lose their homogeneity; at a still later stage septa are formed, but they are far between. In very old cultures they are formed fairly frequently. In these cultures branches have been found to be rather uniformly cut off from their mother

hyphae at the point of origin (Plate V, Fig. 4e). They are generally curved, either concave or convex; they are more or less thickened either on one or both sides by the deposition of cellulose on them. The thickening is either elliptical or conical (Plate V, Fig. 4). It resembles callose caps on sieve plates. The cellulose thickening when stained with Schulze's solution does not take a homogenous blue or violet colour but different shades of blue or violet. This shows that the cellulose on the septa has been deposited in layers. On rare occasions branches have been found to grow through septa (Plate V, Fig. 6). Cellulose ingrowths have been found to form false septa in hyphae (Plate V, Fig. 5). They differ from the ordinary septa in not having their characteristic curve, in being broadened at the ends and not in the middle, and in not being laid down in plates, but in being ingrowths from the inner layer of the hypha walls. The false septa are formed either by two cellulose pegs growing in from the opposite walls and meeting at some point (Plate V, Figs. 4d and 5c) or by cap-like depositions of cellulose on the ingrowths (Plate V, Fig. 5b).

The sporangia are borne on simple stalks, but in water cultures and in certain media the stalks are as irregularly branched as those of *Ph. omnivora* and of *Ph. omnivora*, var. *Areca*. The sporangia are generally apical as in a typical *Phytophthora*; but they are often intercalary and sometimes lateral as well; the end of a hypha swells as if it was forming a sporangium, but for some unknown cause the swelling does not increase and produces three or four short lateral branches, each of which terminates in a sporangium. Occasionally an apical branch also arises from the swelling, which prolongs the vegetative growth of the original hypha. The sporangia are generally pear-shaped but intercalary sporangia are usually round (Plate V, Fig. 1b); they do not vary much in shape but they vary a great deal in size. The variation is between $11-60 \times 10-45\mu$, on an average $25-50 \times 20-40\mu$, and consequently the number of zoospores in them is very irregular. They have from 5 to 45 zoospores, on an average 30. Sporangia and zoospores from diseased leaves are found to be smaller than those from pure cultures. Sporangia have usually one papilla

vertically opposite the stalk, but some sporangia have been found with two (Plate IV, Fig. 36). In such cases the discharge of zoospores does not necessarily take place through the apical papilla.

Sporangia fall off from their sporangiophores with or without any vestige of their stalks. Sporangia from a healthy culture, when suspended in water and in ordinary day-light, discharge their zoospores within five minutes. Sporangia from cultures made in May and June 1912, when the temperature was very high, did not discharge their zoospores so readily. They required to be suspended in water for half an hour to make them discharge their zoospores, but when they were suspended in water and kept in an incubator at 25° C., zoospores were emitted in five minutes. The same result was obtained by adding a small piece of ice to water containing sporangia. Cultures made in these hot months were occasionally daily put in an incubator at 25° C. They produced sporangia luxuriantly, which discharged their zoospores in ordinary tap water or distilled water in five minutes at room temperature. Submerged sporangia from French-bean juice agar and oat juice agar discharge their zoospores even when embedded in the media. These zoospores, after leaving the sporangium crawl about for a few seconds in the film of water expressed from the agar before rounding themselves off, and germinate like other zoospores. Light seems to influence the formation and emission of zoospores. A dozen healthy hanging drop cultures were kept in a dark cupboard and a dozen similar cultures left on the working bench in ordinary day-light. Within half an hour all the mature sporangia in the cultures left on the bench had discharged their zoospores while there was no change in those kept in the dark; but the next day sporangia in these cultures had germinated conidially, giving a highly branched mycelium (Plate III, Fig. 1). Both these sets of cultures were kept under observation for three days. The cultures kept in the dark formed no new sporangia but in those kept on the working bench the sporangia that had not discharged their zoospores had germinated conidially and bore secondary sporangia (Plate IV, Fig. 37); and the new plants got by germination of zoospores had also formed sporangia.

Varying temperature, alternate light and darkness and moisture are essential factors in the formation of sporangia. Cultures kept for months in a dark cupboard at varying room temperature and in incubators at the constant temperatures of 20° C. in summer and 35° C. in winter, produced luxuriant mycelium but remained sterile. But when they were removed to an ordinary room in ordinary atmospheric conditions, sporangia were soon formed. Inoculated castor seedlings kept in a dry place in the laboratory produced no sporangia but those kept in the laboratory compound on misty nights and those kept in the laboratory but occasionally sprayed with water, gave sporangia in forty-eight hours.

An immature sporangium has a single vacuole about 10-12 μ in diameter. It glides from place to place in the sporangium by very slow, almost imperceptible "amoeboid" movements. As it contracts and expands the size of the sporangium has been found to be decreasing and increasing. The formation of zoospores is made faintly evident by the segmentation of the protoplasm into so many units while the vacuole is still present. At a later stage the vacuole disappears and the zoospore origins become more prominent. At this stage they have no vacuoles. They then become more coarsely granular. The sporangium looks rather dark. A little later in each of the zoospores a vacuole is seen appearing and disappearing. The papilla is now very highly refractive. The contents of the sporangium have contracted a little from the inner wall. A slight pulsating movement in it heralds the approaching discharge of the zoospores. There is a slight spasmodic rotation of the whole mass. Individual zoospores are restless, they contract and swell. There is also a slight motion visible in their granular protoplasm. The inner wall opposite the papilla is occasionally pushed into it by a zoospore striking against it. The discharge of the zoospores takes place in either of the following two ways:—

1. The papilla dissolves or bursts under pressure. The zoospores are immediately discharged but sometimes a little granular fluid flows out before them. Sometimes the papilla dissolves before

the zoospores move about in the sporangium or make any effort to escape¹ (Plate IV, Fig. 4).

2. The papilla swells or is blown into a hemisphere by the inrush of the zoospores (Plate IV, Figs. 1 and 2). It disappears before all the zoospores have left the sporangium (Plate IV, Fig. 3). Those that get into it remain stationary, huddled together for a few seconds, before dashing away. The swollen papilla can be distinctly seen by treating a discharging sporangium with iodine. This mode of discharge has been observed in *Pythium palmivorum*.²

The zoospore escapes from the sporangium by contracting its anterior end and squeezing it through the opening; it then swells up, the zoospore becoming dumb-bell shaped. Then the posterior end contracts and passes to the outside. Its vacuole either disappears and re-appears as soon as the anterior end of the zoospore is out of the sporangium, or divides into two, one half going to the front portion and the other to the hinder and both re-uniting when the zoospore is completely out of the sporangium, or along with the contraction of the zoospore the vacuole also contracts, looking like an hour-glass, when the zoospore is partly out of the sporangium. Each zoospore as it makes its escape from the sporangium generally drags out the one behind it. It is not always that the zoospores succeed in getting out of the sporangium. Those that do not find their way out, round themselves off and may germinate *in situ*.³ Zoospores in a sporangium which for any reason get separated from those that are escaping from it, find it difficult, and frequently altogether fail, to get out.

The zoospores, as a rule, escape as distinctly defined units but occasionally in clumps of several united together, moving about by amœboid movements before getting separated from each other.

¹ C.f. Hartig, R. loc. cit., p. 44.

² C.f. Butler, E. J. Bud-rot of Palms in India. Mem. Dept. Agric. India, Bot. Ser. III, No. 4, 1910, p. 254.

³ C.f. Hartig, R. loc. cit. Coleman, L. C., loc. cit., p. 62.

In rare cases two zoospores have been found attached together by a protoplasmic neck (Plate IV, Fig. 6a). The whole mass has been observed to have only two cilia, one at each end. The two zoospores make an effort to get free from each other. They forcibly pull at each other and violently twist round and round the neck, which elongates and consequently becomes thinner. It finally snaps, the zoospores immediately dash away, with a cilium at one end and a protoplasmic thread at the other. This is probably not a formation of two uniciliate zoospores by the fission of one bi-ciliate swarm spore, as found by Atkinson in *Artotroqus (Pythium) intermedius* (de Bary)¹; but merely an incomplete breaking up of the original protoplasm in two units of zoospores. In Atkinson's case the movement of the bi-ciliate zoospore becomes slower five or ten minutes after discharge and "finally it nearly ceases and the body undergoes plastic movements resembling somewhat that of an amoeba. At first this amoeboid movement is irregular, but after a few minutes it assumes a definite character which tends to cut the organism into two." His figures show that the bi-ciliate zoospores have a single vacuole.² What I have found is the appearance of two distinct units connected by a narrow neck at the time of discharge, each unit possessing a vacuole (Plate IV, Fig. 6a); the whole mass being almost twice the size of a normal zoospore; its movement is at no stage "amoeboid," and the two units get separated by the snapping off of the protoplasmic neck, the result of the hard pull that they have at each other. I have found two units thus united together hardly half a dozen times while Atkinson has found the breaking up of bi-ciliate zoospores into two uniciliate ones taking place as a rule. In one case, along with normal zoospores, a motile mass of protoplasm was discharged from a sporangium at 10-15 A.M. (Plate IV, Fig. 26). Its shape was that of a roughly drawn pentagon with a distinct cilium at each of its angles. It whirled round and round a single point as if one of its cilia, which was not visible, had

¹ Atkinson, Geo. F. Damping off, Cornell Univ. Agric. Expt. Stat. Bull. 94, Bot. Division, 1895, p. 249.

² *Ibid.*, pl. II, fig. 24, p. 247.

got entangled with some foreign body. It evidently tried to set itself free. At 10-20 four vacuoles appeared, at 10-25 it succeeded in disentangling itself and suddenly darted off. At 10-30 its motility ceased, it lost its cilia and rounded itself off. At this stage only one vacuole was visible. It measured $15 \times 12\mu$. Its endospore and exospore were quite distinctly visible (Plate IV, Fig. 27). The former was hyaline and refractive, the latter dark. At 10-45 it began to germinate. It produced three germ-tubes. Plate IV, Figs. 27 to 34 show its progress till 3 P.M., after which there was no further development, possibly on account of lack of food. This motile mass of protoplasm, having five visible cilia and going through the ordinary life cycle of a normal zoospore and germinating by giving out three germ-tubes, possibly consisted of three undifferentiated zoospore constituents. Dr. Butler¹ has observed in *Pythium palmivorum* two or more spores remaining united after leaving the sporangium and germinating normally after coming to rest, but they have only a single wall.

The zoospores are of the ordinary *Phytophthora* type and measure $8-12 \times 5-8\mu$ in their motile state, when they come to rest they measure $7-11\mu$. They are bean-shaped with a pointed anterior; arising from the hilum are two unequal cilia, the anterior one being distinctly shorter, as found in *Ph. Syringae*² and *Ph. omnivora*, var. *Arecæ*³ (Plate IV, Fig. 6). After swimming about for twenty minutes to two hours they come to rest. Their movements are very brisk for the first fifteen minutes; they move about even after assuming the spherical shape, the movement then being not very energetic. The rounded zoospore after rolling about for a few seconds comes to a dead stop to all appearances, but quite suddenly after a few seconds it dashes away, presumably in search of another suitable resting place. This fitful and sudden movement goes on for about five minutes more and then the spore comes to a dead halt. In fifteen minutes germination takes place

¹ Butler, E. J. Bud-rot of Palms in India. Mem. Dept. Agric. India, Bot. Ser. III, No. 4, 1910, p. 255.

² Klebahn, H. *loc. cit.*, p. 51.

³ Coleman, L. C. *loc. cit.*, p. 63.

generally by one and rarely by two germ-tubes (Plate IV, Figs. 8 to 25). The germination does not seem to be affected by light or darkness. Two sets of experiments were carried out to see the action of light on the germination of zoospores. A little bit of mycelium from a pure culture was transferred to tap water. Within ten minutes an army of zoospores was actively moving about. They were transferred to twelve slides, half of which were kept in a moist chamber exposed to ordinary day-light at laboratory temperature, while the rest were kept in a moist chamber in a dark cupboard at the same temperature. In two hours all the spores had germinated equally well in both the sets.

The germination of the sporangia takes place in three different ways. (1) When they are placed in a drop of fresh water their protoplasmic contents break up into the constituent zoospores, which after escaping from the sporangia germinate readily within half an hour. (2) They may give rise to one or more short germ-tubes forming secondary sporangia, which may either form zoospores or tertiary sporangia at the end of short germ-tubes. It is not unusual to find three or four generations of sporangia linked together by short germ-tubes (Plate V, Fig 1). This mode of germination is generally found in old cultures and in those not kept in pure water. (3) They germinate conidially giving rise to a mycelium.

In cultures and on inoculated castor fruits a second spore form has been found, the "resting" conidium or so-called "chlamydospore." This "resting" form of conidium has been observed in the Cacao *Phytophthora* by Rorer¹ and apparently also by Petch², and in *Pythium palmivorum*³ by Dr. Butler. The "resting" conidia are usually spherical, thick and smooth walled, yellow in colour, terminal, lateral or intercalary, 20-60 μ in diameter and contain a lot of oil globules (Plate V, Figs 1a and 7 to 13). Intercalary "resting" conidia are formed in two ways. (1) In the course of a hypha, swelling takes

¹ Rorer, J. B. *loc. cit.*, p. 13.

² Petch, T. Cacao and Hevea Canker, Gr. and Agric. Journ. Royal Bot. Gard., Ceylon, V, No. 13, 1910, p. 153.

³ Butler, E. J. *loc. cit.*, p. 255.

place in both its walls or in one at first, forming a round or elliptical enlargement which is then cut off by two septa (Plate V, Figs. 11 and 13). The thickening of the wall begins at a very early stage. (2) A terminal swelling appears on a young hypha, which soon becomes cut off by a septum from the swelling; the growth of the hypha is continued either in a prolongation of its original course or in a new direction by the swelling putting out a new hypha. This is not a germination of the swelling by a germ-tube, but it is merely a vegetative growth as the swelling is immature and develops into a fully mature thick-walled yellow "resting" conidium after the new hypha has grown out. It is capable of germinating independently. In cultures "resting" conidia are borne both on the aerial and submerged mycelium. They are formed both in healthy and unhealthy cultures. Extremes of temperature, which retard the formation of sporangia, do not retard the formation of "resting" conidia. Cultures made in May and June last (1912) produced a poor crop of sporangia on account of the excessive heat in these months but were rich in "resting" conidia. Very old cultures, about nine months old, in which all sporangia and hyphae were dead, contained living "resting" conidia. The "resting" conidia do not necessarily require a period of rest before germinating. They germinate by one or more germ-tubes quite readily (Plate V, Fig. 8). When "resting" conidia are sown in water, germination takes place within twenty-four hours. Very frequently the germ-tubes are swollen at the base. The germ-tubes either directly produce sporangia (Plate V, Fig. 12) or "resting" conidia (Plate V, Fig. 10) or vegetative hyphae. If kept moist they retain their vitality for over nine months, but if they are completely dried, their germinating power does not last for even a week.

That the "resting" conidia are parthogenetic oospores is very improbable for the reason that they are over twice the size of normal oospores and that from the very beginning of their development they are thick-walled and slightly yellow tinted, while parthogenetic oospores have their origin in thin-walled and hyaline oogonia which, on failing to come in contact with antheridia

after reaching maturity, undergo the same changes, at least outwardly, as they would have if they had been fertilised. The "resting" conidia germinate readily, while the oospores have not been found till now to germinate, except in one doubtful case.

Von Faber¹ in Cacao *Phytophthora* has found oospores but he failed to find antheridia and oogonia. Coleman² explains this failure on the ground that the oospores "almost always fill the oogonial cavity so completely that the oogonial wall can be made out only with difficulty." His figures 7, 8, and 9 of Plate XVIII leave doubts in my mind as to their being drawings of true parthogenetic oospores. They seem to be "resting" conidia or chlamydospores, which Rorer has found in diseased fruits. They quite resemble "resting" conidia found in *Ph. parasitica*.

In the *Phytophthora* on *Hevea brasiliensis*, which is considered to be *Ph. Faberi* by Petch and others, oospores have been found but "Oogonienhäute und Antheridien waren nicht vorhanden."³ From spirit specimens of fruit of *Hevea brasiliensis* attacked by *Nectria* and by *Phytophthora* I have come across globular yellow thick-walled (2μ thick) bodies, in appearance exactly similar to "resting" conidia found in *Ph. parasitica*. They measure 22 to 30μ in diameter, on an average 27μ . These "resting" conidia might have been mistaken as oospores without antheridia and oogonia.

As in *Ph. Phaseoli*⁴, the antheridium is the first to be found in the development of the sexual organs. The formation of the antheridium is indicated by the terminal swelling of a hypha, which sometimes prolongs its course to a few microns in length beyond the swelling, or by the swelling of the wall of a

¹ Von Faber, F. C. Die *Phytophthora*-Fäule der Kakaofrüchte, Arbeiten aus der Kais. Biol. Anst. für Land- und Forstwirtschaft, VII, 1910, p. 201.

² Coleman, L. C. loc. cit., p. 74.

³ Peters, Über eine Fruchtfaule von *Hevea brasiliensis* in Kamerun. Ber. üb. d. Tätigkeit d. Kais. Biol. Anst. im Jahre 1911. Mitt. Kais. Biol. Anst. 1912, No. 12.

⁴ Clinton, G. P., loc. cit., 1907-1908, p. 902.

rather old hypha somewhere in its middle. Thus according to its development the antheridium is terminal or intercalar. The swollen portion of the hypha is soon cut off by one or two septa according as it is terminal or intercalar. The walls of the swelling remain thin or become slightly thicker than those of its stalk. There is no trace of the oogonium till the antheridium is completely developed, or almost so. The oogonium and the antheridium are on the same stalk or on different stalks. When they are on the same stalk the oogonial hypha arises from the base of the antheridium as an ingrowth (Plate VI, Figs. 3 and 4). When the oogonial origin is developed on a separate hypha, it grows towards the antheridial swelling and makes its way into the interior of the latter when it comes in contact with it. In some cases the oogonial hypha swells before penetrating the antheridium (Plate VI, Fig. 14), in rare cases it has been found to indent into the wall of the antheridium before boring its way through it (Plate VI, Fig. 2). Once within the antheridium the hypha continues its growth until the apex is reached. At this stage the swollen apical wall of the oogonial hypha is thinner than its side walls (Plate VI, Figs. 4 to 7), and its apex is also more highly refractive than the rest of the hypha. The swollen head of the oogonial hypha now dissolves the antheridial wall in contact with it and grows out of it. Immediately it emerges it, as a rule, swells out into a sphere or almost a sphere containing oil drops, the oogonium proper. When the oogonium is mature its protoplasm gets differentiated into the oosphere, surrounded by a very thin hyaline wall. Later on changes take place in the oosphere, the thin wall begins to get thickened and contracts a little from the oogonial wall. There is a simultaneous change in the oogonial wall as well; it also gets thickened and turns yellow. These changes mark the conversion of the oosphere into the oospore, after fertilisation, but the exact moment of fertilisation cannot be ascertained; at no stage in the development of the antheridium and oogonium has ever been found any special process through which the fertilisation might be effected, probably on account of the way in which the antheridium envelops the oogoni-

um. They are in such intimate contact over a considerable area that nuclear transfer might take place at any time after the penetration of the antheridium by the oogonial origin. After fertilisation, the oogonial stalk within the antheridium is found in the majority of cases to possess a septum or a thick cellulose plug or both; in those cases in which neither septa nor cellulose plugs have been observed, they might be present just where the antheridium is pierced by the oogonial stalk and so escape observation. The oogonium forms a permanent coating round the oospore which almost fills it; even after the formation of the oospore the antheridium is not always completely empty of its protoplasmic contents.

I cannot definitely say if the oogonium is ever developed terminally, but in those few cases in which the hyphae bearing the oogonium could be clearly traced for some distance, it was found that it was on a lateral branch of another hypha (Plate VI, Figs. 10, 15 and 20), or occasionally sessile or what may be termed intercalary (Plate VI, Fig. 11).

Both Jones and Clinton¹ have observed that the oogonial thickening in *Ph. infestans* is influenced by the medium in which it is grown. A similar difference in the oogonial thickenings of the oospores of *Ph. parasitica* is found in French-bean juice agar and oat juice agar. The oogonium in the former medium is slightly thickened, at times very little, smooth and yellow. Within it is seen the oospore, almost filling the oogonial cavity. Very often the enclosed oospore lies in such close contact with some portion of the oogonial walls that it is very difficult to differentiate between their walls at this place. The oogonium measures 15-27 μ in diameter, as a rule 18-25 μ , and the average of 60 measurements gave a diameter of 23.8 μ . The oogonium in oat juice agar is thick-crusted on account of secondary thickening on its wall, orange yellow in colour, and without a sharp contour. At times the thick oogonial wall is so opaque that the enclosed oospore which almost fills the oogonium is only seen on crushing it. These thick-walled dark

¹ Clinton, G. P. *loc. cit.*, 1909-1910, p. 772.

coloured oogonia growing in oat juice agar, look similar to those of *Ph. infestans* found in the same medium by Clinton¹, but they are much smaller. The thick-walled oogonia of *Ph. parasitica* vary from 25 to 35 μ in diameter, while those of *Ph. infestans* vary from 34 to 50 μ in diameter. Though the oogonia from French-bean juice agar are smaller than those from oat juice agar, this difference in size being due only to the nature of the two media, still the oospores from both these media are of the same size. They vary from 13 to 24 μ in diameter as a rule from 15 to 20 μ . The average of 60 measurements gave a diameter of 18.6 μ . They have a thick wall, about 1 to 2 μ in thickness, and are spherical, smooth and hyaline. They have a homogeneous mass of protoplasm, generally containing one or more oil globules.

The antheridia are hyaline, thin-walled and irregular in shape. Those produced in French-bean juice agar are larger than those found in oat juice agar. They are persistent; even in very old cultures they have been found to remain attached to the oospore. The oospore breaks off from its antheridium when roughly manipulated.

The oospores in oat juice agar are embedded in the medium and are, as a rule, confined to those portions containing matted budding hyphae, which are considered to store reserve material for the fungus. These are invariably empty after the formation of the oospores. Very often the oospore is completely entangled in the network of those hyphae. The oospores in French-bean juice agar are embedded in the medium, but they are not restricted to any particular portion of it. They are found mixed up with sporangia and "resting" conidia.

The oospores of *Ph. infestans* "are more likely to be found in the upper and drier part of the tube"² but the oospores of *Ph. parasitica* have invariably been found in the lower and moister part of the tube and never in the upper and drier. There is as much variability and uncertainty in the production of these oospores as Clinton found in the production of those of *Ph. infestans*. Some

¹ Clinton, G. P. *loc. cit.*, 1909-1910, p. 772.

² *Ibid.*, p. 765.

portions of the culture tube contain a lot of them in all stages of development, while in other portions there is no trace of them.

Ph. parasitica oospores have been found in French-bean juice agar and oat juice agar but they have only been obtained when these media were inoculated from cultures growing on a different medium.

Clinton has found it difficult to decide whether the oogonial thread "did not actually penetrate the antheridium and grow through it," but "the optical effect was frequently that of an internal thread." I have not found a single case in which the oogonial origin was not within the antheridium. The microphotographs bear independent evidence to the unique mode of development of the oogonium. One of them (Plate VII, Fig. 1) shows the oogonial thread arising as an ingrowth within the antheridium. Another (Plate VII, Fig. 5) shows a roughly manipulated oospore. At the apex of the antheridium the elliptical opening made by the oogonial thread was distinctly visible. Within the antheridium is seen the passage made by the oogonial thread in the course of its upward progress. Plate VI, Fig. 16, shows an unfertilised oogonium which through rough handling got a little separated from its antheridium. This figure clearly illustrates the exact origin of the oogonium.

It is a matter of common observation that when a hypha penetrates its host cell at right angles to it, under the microscope the penetrating end of the hypha, observed vertically, appears as a continuous chain of rings lying within the cell when the fine adjustment of the microscope is being used. The same phenomenon is observed in the antheridium which is being pierced by an oogonial hypha (Plate VI, Fig. 1). When the oogonium and antheridium are on the same hypha the oogonial stalk broadens out at the place of its origin at the base of the antheridium. All these observations leave no doubt as to the oogonium arising from within the antheridium.

COMPOSITION OF THE MEMBRANES.

According to Mangin¹ the mycelium of the *Peronosporaceæ* can be very easily distinguished from other fungi by the "constant presence of callose, pure or associated with cellulose" which partially or completely plugs the mycelial tubes at intervals. I have failed to find this constant presence of callose in *Ph. parasitica*. No doubt within the hyphæ have been seen refractive, homogeneous, hyaline and generally bi-convex bodies which completely fill the hyphal cavity (Plate V, Figs. 5a, c and d). They arise as ingrowths from the inner walls of the hyphæ (Plate V, Figs. 1, 4d and 5). The hyphal cavity is closed either by one such ingrowth extending from one wall to the other or by two or more arising from the opposite sides and meeting at some point within the hypha. These plugs, unlike the callose plugs found by Mangin² in the other *Peronosporaceæ*, have never been observed to originate as annular rings within the hyphæ. They are composed of pure cellulose and not of pure callose or callose associated with cellulose. The presence of callose is indicated by a yellow colour given to it by the iodine reagents for cellulose, *e.g.*, calcium chloride-iodide, iodine and phosphoric acid, iodine and sulphuric acid and Schulze's solution. These reagents have invariably stained the plugs blue or violet without any trace of yellow. Caustic soda and caustic potash easily dissolve callose; ammonium and alkali carbonates swell it and give it a gelatinous appearance; sulphuric acid slowly dissolves it; cupric ammonium hydrate has no action on it even after long treatment with strong acids. These are the reactions given by Mangin³ for callose. The plugs found in our fungus are not dissolved by caustic soda or caustic potash; they are not affected by ammonium and alkali carbonates, they are readily dissolved by sulphuric acid; after two hours' treat-

¹ Mangin, L. Sur la structure des *Péronosporées*. C. R., CXI, 1890, p. 924 and Recherches sur les *Péronosporées*. Bull. Soc. Hist. Nat. d'Autun, VIII, 1895, p. 76.

² *Ibid.* Sur la désarticulation des conidies chez les *Péronosporées*. Bull. Soc. Bot. Fr., XXXVIII, 1891, p. 234.

³ *Ibid.* Sur la callose, nouvelle substance fondamentale existant dans la membrane des cellules des végétaux. C. R., CX, 1890, p. 646.

ment with hydrochloric acid, cupric ammonium hydrate dissolves them completely in a short time. Thus the reactions which helped Mangin to trace the presence of callose, pure or associated with cellulose, in the *Peronosporaceæ* examined by him, have shown us the complete absence of the substance from the plugs found in *Ph. parasitica*. These plugs give all the colour and chemical reactions for cellulose. Mangin has not only found callose plugs in the mycelium of the *Peronosporaceæ*, but also callose depositions on the walls. These depositions as well have been given great importance by him, as being an important distinguishing characteristic of the order. The mycelium of *Ph. parasitica* has been found studded on its inner walls with some deposit which gives all the chemical and colour reactions of cellulose and none of callose. These cellulose deposits are very beautifully seen if the mycelium be first treated with a strong acid, such as phosphoric acid, for a few hours and then treated for a very short time with a weak solution of cupric ammonium hydrate and stained with phosphoric iodide after being well washed with water. The mycelium takes a beautiful blue colour and the studs on it a darker blue, without any trace of yellow in them. This studded mycelium looks quite similar to what is figured by Mangin.¹ This studded deposit of cellulose has also been found in sporangia and "resting" conidia. Mangin has observed no deposit on the sporangia of the *Peronosporaceæ* examined by him, at least he makes no mention of them in his various notes on this subject I have consulted. Callose has been found to be absent even from the septa. The beginning of the formation of the septum is seen as a hazy, ill-defined, hyaline and slightly refractive line, running from one wall to the other in the midst of a mass of protoplasm. When stained with iodine and phosphoric acid the septum stands out clearly by its faint blue colour in the yellow coloured protoplasm. This hazy and ill-defined line, later on becomes sharply defined and stains deep blue with iodine and phosphoric acid. Cellulose thickening then

¹ Mangin, L. Recherches sur les *Péronosporées*. Bull. Soc. d'Hist. Nat. d'Autun, VIII, 1895, fig. 8, p. 88, and fig. 9, p. 90.

begins to take place on the side or sides where the protoplasm is present. If the protoplasm disappears from both the new cells formed by the septum there is no thickening on it. This thickening of the septum is found to be of pure cellulose. It is dissolved by cupric ammonium hydrate and sulphuric acid, no way affected by caustic potash, alkali carbonates and ammonia and stains blue or violet with all the cellulose iodide reagents. This thickening on the septum is found not only in the vegetative hyphæ but also on the septum of the oogonial stalk within the antheridium and on the antheridial septa as well.

These cellulose plugs in hyphæ, and cellulose deposits on their walls and septa, have been observed by me in the hyphæ of *Ph. Faberi* also.

The sporangia are composed of pure cellulose, except their papilla. As already stated they have on their walls irregular cellulose thickenings. At the point of attachment to the sporangiophores generally a thickening has been found, which is of pure cellulose and not of callose as observed by Mangin¹ in the other *Peronosporaceæ*.

The "resting" conidia have been found to possess three walls; the outer, thin and hyaline, is closely applied to the central thick and yellowish wall and escapes observation unless it is stained; the innermost wall is thicker than the outer but thinner than the central wall, its colour is masked by the yellow of the latter, to which it is not closely attached. Very young "resting" conidia apparently stain yellow with the iodide reagents for cellulose, but if they be closely examined a faint coat can be detected around them. In very young intercalary "resting" conidia, which have not become spherical but are ellipsoidal, a quantity of cellulose is collected at the ends. When mature the "resting" conidia colour blue or violet with iodine and phosphoric acid or with any other iodine cellulose reagent, as the outer wall has become thicker. If they be first treated with hydrochloric acid for a couple of hours and then for a short

¹ Mangin, L. Sur la désarticulation des conidies chez les *Peronosporacées*. Bull. Soc. Bot. Fr., XXXVIII, 1891, p. 234.

time with cupric ammonium hydrate the outer wall is dissolved. After this treatment iodine and phosphoric acid stains the central thick wall quite yellow and the interior of the conidium appears filled with a blue mass, possibly the dissolved inner wall. If in place of strong cupric ammonium hydrate a weak solution of this reagent is used for a short time, remnants of the outer thin wall are visible and they stain blue with the cellulose colour reagents. Evidently the thicker central yellow wall is of quite a different chemical composition from the other two, which are shown to be of cellulose. Ruthenium red, acidified Bismark brown and alum hæmatoxylin stain the "resting" conidia not very sharply without any previous treatment, on account of the outer coat of cellulose on which these stains have no effect. But when the outer wall is dissolved by cupric ammonium hydrate, acidified Bismark brown stains the central wall brown, which colour is not washed out by hydrochloric acid, while alum hæmatoxylin stains it violet blue very clearly. Mangin gives these stains as specific reagents for compound pectic substances. Ammonia and alkali carbonates have not any action on the thick yellow coloured central wall and therefore it is not composed of pure pectose, but of some compound pectic substance. When treated with sulphuric acid the central wall remains undissolved, even after a long treatment, while the two other walls are completely dissolved in a short time; caustic potash does not immediately dissolve it. These reactions clearly prove the absence of callose from the walls of "resting" conidia.

The composition of the antheridial wall is quite simple. It is constituted of pure cellulose without any callose. It is immediately dissolved by sulphuric acid and cupric ammonium hydrate: ammonia, caustic alkalies and caustic carbonates have no action on it; it readily stains blue with all the cellulose colour reagents.

The walls of the unfertilised oogonium are composed of pure cellulose. After fertilisation the oogonial wall undergoes modification. In the *Peronosporaceæ* investigated by Mangin¹, the

¹ Mangin, L. Recherches sur les *Péronosporées*. Bull. Soc. d'Hist. Nat. d'Autun, VIII, 1895, p. 99.

oogonium at this stage was found to possess two walls; the internal contained only cellulose and no callose, or if at all very little, and the external, on the contrary, contained chiefly callose and little or no cellulose. In *Ph. parasitica* it is also surrounded by two walls but the outer thick wall is not found to be of callose. Congo red,¹ which stains both cellulose and callose, failed to stain the oogonium, though hyphæ, sporangia, "resting" conidia and antheridia were stained. Cold strong sulphuric acid had no action on it, even though the treatment was prolonged for over a week, alkali carbonates and ammonia neither swelled it nor gave it a gelatinous appearance, iodide reagents for cellulose intensified its original yellow colour, it was not stained by nigrosin. The outer thick wall of oogonia of *Sclerospora graminicola* has been found by Mangin² to consist of pure callose and under the influence of phosphoric acid this thick wall swells excessively, becomes jelly-like and sets free the oospore, but the oogonial wall of *Ph. parasitica* is not affected by this acid, even after a long treatment. Malachite green, gentian violet, carbofuchsin stain deeply all the parts of the fungus but the stain is washed away by the use of acidulated water from all of them, except from the oogonium. The same selective affinity for the oogonium is shown by Hofmann's violet. Safranin, Kleinenberg's Hæmatoxylin, Delafield's Hæmatoxylin and eosin do not stain the oogonium; iodide reagents for cellulose only intensify its yellow colour. These chemical reactions and stains show that this thick outer wall is composed of a chemical substance other than callose or cellulose.

The possibility of this thick wall being composed of some pectic compound substance was suggested by its staining beautifully red with Ruthenium red, which has been successfully used by Mangin³ for detecting the presence of compound pectic substances in the

¹ Mangin, L. Sur la callose, nouvelle substance fondamentale existant dans la membrane des cellules des végétaux. C. R., CX, 1890, p. 645.

² *Ibid.*, Recherches sur les Péronosporées. Bull. Soc. d'Hist. Nat. d'Autun, VIII, 1895, p. 97.

³ *Ibid.*, Sur l'emploi du rouge de ruthénium en Anatomie végétale. C. R., CXVI, 1893, p. 653.

higher plants. He considers this to be the only reagent for the products of the transformation of compound pectic substances. They are dissolved by the successive action of hydrochloric acid and an alkali. As recommended by Mangin¹ the oospores were at first boiled for half an hour with two per cent. hydrochloric acid and then with two per cent. caustic potash for a long time; after each boiling they were thoroughly washed. The result was that the outer thick coat of the oogonium was dissolved, exposing the thin inner coat, which had swelled under the treatment. The oospore was lying loosely in it. The sporangia and "resting" conidia had considerably swelled. Iodine and phosphoric acid and the other iodide reagents of cellulose stained blue or violet the swollen internal wall of the oogonium. Eau de javelle completely dissolved the outer thick wall in twenty-four hours. After treatment for an hour or two, it became quite soft and allowed the cellulose stains to reach the internal wall and the oospore. The outer thick coat stained brown with acidified Bismark brown and the stain was not washed away by acids and alcohol; it also stained violet with alum hæmatoxylin. The former reaction shows that it is neither lignified nor suberised, for the stain given to lignin and suberin by Bismark brown is washed out by acids and alcohol. When treated with seven per cent. caustic potash for a week or so the outer coat is completely dissolved and the inner coat is also completely dissolved or almost so; in the latter case merely a faint halo is visible round the oospore. This halo becomes just visible by its taking a bluish tinge with phosphoric iodide. This inner wall, therefore, is probably composed of some soluble form of cellulose, similar to that which has been found by M. Hofmeister² to stain with zinc-chloride-iodide and to be soluble in five or six per cent. of alkali.

The composition of the outer coat, which is dissolved by seven per cent. caustic potash after a long treatment, by eau de javelle in twenty-four hours and by the successive treatment with hydro-

¹ Mangin, L. Nouvelles observations sur la membrane. Bull. Soc. Bot. Fr., XI, 1893, p. 273.

² Vide Mangin, L. *loc. cit.*, p. 278.

chloric acid and caustic potash, which is not dissolved by acids, alkali carbonates or ammonia, which stains red with Ruthenium red, violet with alum hæmatoxylin,¹ and brown with acidified Bismark brown,² the stain being not washed out by acids and alcohol, is presumably some compound pectic substance.

The stalk of the oogonium within the antheridium undergoes the same modification as the fertilised oogonium, though not to such a great extent as the latter. This stalk occasionally has a cellulose plug like those found in vegetative hyphæ.

The composition of the walls of the oospore could not be accurately determined. But the outer wall appears to be composed of some compound pectic substance and the inner wall of cellulose; because the solvents of the outer wall of the oogonium dissolved the outer wall of the oospore and the undissolved part of it gave all the reactions of cellulose; because strong sulphuric acid did not dissolve the outer wall of the oospore; again when the oogonium was allowed to be acted upon either by weak solutions of its solvents or by strong solutions for an insufficiently short time the enclosed oospore when treated with the iodide reagents of cellulose stained yellow on the outside and blue in the interior.

During this work I have found chlor-zinc-iodide and iodine and sulphuric acid, commonly used for staining cellulose, to be very unsatisfactory as their action is capricious, but calcium-chloride-iodide and iodine and phosphoric acid recommended by Mangin have given excellent results, especially the latter; with its use the intensity of the stain can be easily regulated by the use of different strengths of iodine solution and the stained slides have been found to keep well for over two months.

MEDIA.

French-bean (*Phaseolus vulgaris*) juice agar and oat juice agar have proved to be the best media. These two media have been

¹ Mangin, L. Sur la constitution de la membrane des végétaux, C. R., CVII, 1888, p. 145.

² *Ibid.*, Sur la présence des composés pectiques dans les végétaux, C. R., CIX, 1889, p. 580.

found to be equally successful for cultures of *Ph. Faberi*. Oospores of the castor *Phytophthora* were produced in these media quite luxuriantly, especially in French-bean juice agar. Both these media give a very poor growth of the fungus if they are older than about two months. It has been found advisable to use freshly made cultures. In old media the fungus grows very slowly and produces very scanty aerial mycelium, more scanty sporangia and rarely oospores.

The acidity of French-bean juice agar and of oat juice agar has been found to be +2 Fuller's scale for each. Of course there must have been slight variations in the different sets of these media prepared at different times. The following notes give a brief account of the growth of *Phytophthora* from *Ricinus* on the various media tried.

French-bean (Phaseolus vulgaris) juice agar (50 + 10 + 500).— Soon after inoculation, the growth was confined to the surface of the medium; in a couple of days a rich aerial growth of the mycelium was produced which eventually filled the whole tube. The hyphae of the aerial mycelium remained unbranched for long distances; when observed in a mass they presented a regular even surface. The hyphae on the surface of the medium showed attempts at branching, most of which were abortive, hence an irregular thorny appearance was given to these hyphae. The submerged mycelium was profusely branched. It also contained irregularly swollen hyphae. The aerial and submerged mycelium produced a rich crop of sporangia and "resting" conidia. As we have seen oospores were luxuriantly formed, embedded in this medium, but only when it was inoculated with the fungus grown on another medium. This medium was prepared in almost the same way as Lima-bean juice agar by Clinton.¹ Fifty grammes of dried beans were powdered in an iron pestle and mortar, made up with about 300 c.c. of water, then either simmered for half an hour or cooked in the steam of a Koch's sterilizer for half an hour. The latter course was preferable as in the former case there was a likelihood of cracking the vessel if it were of glass or of charring the pounded beans if it were

¹ Clinton, G. P. *loc. cit.*, 1907-1908, p. 898.

a metal one, as the powder did not always get thoroughly wetted by the water used. The liquid was then strained off through a fine wire gauze strainer, and 10 grammes of agar melted in a small amount of water were added to it, the medium was then made up to 500 c.c. by the addition of the requisite amount of water. It was then boiled to thoroughly mix its constituents, strained again through a fine piece of cloth into test tubes and autoclaved.

Oat juice agar (50 + 10 + 500).—Growth at first was not as rapid as on French-bean juice agar. But later on the aerial mycelial growth was as good as in the latter. Both the aerial and submerged mycelium resembled those in French-bean juice agar, but the budding hyphae of the submerged mycelium were more prominent in this medium, especially near about where oospores were formed. Sporangia and "resting" conidia were freely formed. Oospores were not as luxuriantly produced as in French-bean juice agar. Even in this medium they were found only when it was inoculated with a culture grown on another medium. Stab cultures produced a very poor growth, both of the vegetative and reproductive organs. This medium Clinton found to be successful for the formation of oospores of *Ph. infestans*.¹

Clinton gives a very elaborate method for preparing this medium but our simplified method has given us a medium in which oospores have been fairly richly formed. Fifty grammes of crushed oats such as are fed to cattle on the farm were cooked with about 300 to 350 c.c. of distilled water for half an hour in the steam produced in a Koch's sterilizer. The cooked material was then strained through a wire gauze strainer; to the liquid was added 10 grammes of agar dissolved in a sufficient quantity of distilled water to bring it up to the required 500 c.c. The medium before being poured into test tubes was boiled again for a short time in order to thoroughly mix the ingredients. The test tubes were then autoclaved.

Maize corn meal agar (50 + 10 + 500).—This medium was prepared by boiling the meal in water and mixing it with agar dissolved in a

¹ Clinton, G. P. *loc. cit.*, 1909-1910, p. 760.

sufficient quantity of water to bring the whole up to 500 c.c. The mixture was brought to the boil and strained through linen before filling the tubes. The aerial growth was copious but at first sterile, sporangia were formed after a week. Submerged hyphæ were irregularly swollen.

Maize corn meal.—This medium was prepared by adding to the meal a sufficient quantity of water to keep the medium moist after it was autoclaved. The fungus growth was very good. A rich crop of sporangia was obtained.

Potato juice agar (150 + 10 + 500).—Growth in this medium was not localised; it was mostly in the substance of the medium and there was a very sparse aerial growth. The submerged mycelium differed from the aerial in being broader and irregularly branched. Sporangia were formed freely both in the aerial and submerged mycelium but none of these were found to produce zoospores. They germinated conidially without falling off from their conidiophores. They were abnormal both in size and shape and contained a lot of oil globules; an extreme measurement was 110 to 35 μ (Plate V, Fig. 14), but when a bit of the mycelium was transferred to water normal sporangia were produced in twenty-four hours. "Resting" conidia were liberally formed.

Boiled rice.—For the production of aerial mycelium no medium except wheat meal was found to be better. The mycelium did not produce irregularly branched or budding hyphæ. At first only a sterile growth was obtained, but about a month later a few sporangia and many "resting" conidia were produced. When a sterile culture was incubated at about 18°C. a very poor crop of sporangia was produced within a couple of days, possibly on account of the sudden change of temperature. When a bit of the sterile mycelium was transferred to water, sporangia were soon formed.

Wheat meal.—This was prepared in the same way as maize corn meal. It proved superior to boiled rice as it gave a more copious aerial mycelium which readily produced sporangia in abundance.

Bread paste (+10 *Fuller's scale*).—This medium was prepared by mixing powdered stale bread with a sufficient amount of

water to keep it moist after sterilization. Aerial growth was as good as on maize corn meal. A very poor crop of sporangia was produced after a fortnight, but many "resting" conidia were formed. Sporangial growth was as poor in the -5 Fuller's scale bread paste. In neutral and +5 Fuller's scale media, sporangia were produced in a fairly good quantity.

Ricinus leaf juice.—This medium was made by boiling about 15 grammes of green leaves in 100 c.c. of water. The growth was poor, the mycelium had no irregularly budding hyphæ. No sporangia were produced.

Ricinus leaf juice agar (100 + 7 + 500).—The growth was submerged and localised. Vegetative hyphæ branched in a coral-like fashion. Sporangial formation was very poor.

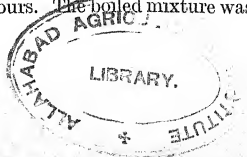
Ricinus seed juice agar (12 + 5 + 500).—This medium was prepared as follows: 12 grammes of seeds, with their hard seed coats removed, were slightly crushed and boiled in about 100 c.c. of water. To the filtered decoction was added 5 grammes of agar, dissolved in water and the whole mixture was made up to 500 c.c. The fungus grew moderately well on this medium. Growth was concentric round the point of inoculation and it was entirely submerged. The vegetative mycelium had only a few swollen or budding hyphæ. Sporangia were copiously produced.

Peptone water (Dunham's solution).—There was a moderately diffused mycelial growth. No sporangia were formed.

Peptone Bouillon.—The growth was not so good as in Peptone water; the vegetative mycelium contained abnormally broad and budding hyphæ.

Peptone broth.—The fungus grew very poorly on this medium. Vegetative hyphæ were irregularly branched and budding. Sporangia were formed. The mycelium growing within this medium and on several other media, resembled the sterile, aquatic mycelium of *Pythiacystis citrophthora*, Smith and Smith, grown in dilute prune juice agar (Plate V, Fig. 16).

Groundnut agar.—Twenty seeds of groundnut powdered in a mortar were boiled in 150 c.c. water for two hours. The boiled mixture was



allowed to stand for 24 hours. The supernatant liquid was filtered, mixed with 7.5 grammes of agar dissolved in water and was made up to 500 c.c. The mycelium radiated from the point of inoculation. The growth was fairly good but submerged. The vegetative hyphæ were broad and budding and branched at right angles. They were full of large oil granules. They presented a warty appearance on account of many of the branches failing to make further progress after they had originated. They were at some places so broad that they looked as if they were galled. Sporangia were few and scattered.

Jowar (Sorghum vulgare) agar.—The medium was made up in the same way as groundnut agar except that 1 ounce of well ground jowar grains were used instead of groundnut seeds. The culture on this medium was in every way the same as on groundnut agar, except that there was a little aerial mycelial growth and that the sporangiferous hyphæ also branched and budded like the vegetative hyphæ but not so much.

Potato slab.—The aerial growth was quite luxuriant but was never found to be fertile. The inoculation succeeded only on the cut surface and not on the peel, just as in the living potatoes.

French-bean powder.—The aerial growth was fairly good. Very few sporangia were produced but there were many "resting" conidia.

Glucose meat-extract agar.—This medium consisted of :—

Extract of Lemco	2	grms.
Sodium chloride	2.5	"
Peptone	5	"
Glucose	10	"
Agar	7.5	"
Water	500	c.c.

The growth was wholly within the medium and not localised. The fungus gave the surface of the medium a roughened appearance as if it had bacterial contamination. The hyphæ were fertile, irregularly branched and swollen as in groundnut agar and other media.

Meat-extract gelatine.—The growth was very poor. No sporangia were produced.

Dolichos live beans.—The growth of the mycelium was over one-third inch high. Sporangia and "resting" conidia were formed in abundance.

Prune juice agar.—This medium was prepared by boiling prunes in 100 c.c. water for 5 minutes and adding to the filtered decoction 7.5 grammes of agar dissolved in water, making up the whole mixture to 500 c.c. The growth was not very poor. The mycelium contained irregularly swollen budding hyphæ. Sporangia were poorly formed.

Prune juice agar acidified with citric acid.—On this medium the fungus grew fairly well but the mycelium remained sterile even one month after inoculation. The hyphæ did not contain irregular branches or abnormal swellings.

Sterilised slabs of carrot, sweet potato (*Ipomoea batatas*) and of corms of *Colocasia antiquorum* did not give the slightest growth of the fungus.

Cultures were also made on sterilised flies and ants in sterilised water but they gave a very poor growth of hyphæ and sporangia.¹

INOCULATION EXPERIMENTS WITH, AND AFFINITIES OF, *Ph. parasitica*.

In order to test the affinity of *Ph. parasitica* with the various species of this genus, a number of inoculation experiments were undertaken on as many of their host plants or their allied species as were available. Inoculations were invariably done by means of suspensions of motile zoospores in distilled water taken from pure cultures. The inoculated plants, seedlings and cuttings were covered with bell jars and the inoculated fruits were placed in moist chambers to keep them constantly in a moist atmosphere. The "checks" were kept in exactly similar atmospheric conditions.

Fruits of Cacao, two species of *Oenothera* and of *Cereus* were obtained through the courtesy of Mr. C. Mahaluxnuwalla, Superin-

¹ Cf. Coleman, L. C. loc. cit., p. 71.

tendent of the Victoria Gardens, Bombay ; plants of lilac were kindly supplied by the Superintendent, Kumaun Government Gardens.

Young plants of *Solanum tuberosum* were inoculated with zoospores. The plants showed signs of disease in twenty-four hours. The leaves turned black and shrivelled up as if attacked by *Ph. infestans*, and the plant wilted (Plate VIII A). No sporangia were formed on the diseased parts but if they were transferred to water sporangia were readily produced. When a very tender stem was inoculated, from the diseased area a web of aerial sterile mycelium was produced. The diseased area extended both ways. A thick or an old stem took no inoculation, even when wounded. The disease remained localised on inoculated mature leaves. Tubers could be inoculated only through wounds. They produced a rich aerial growth of mycelium but this was always sterile.

Seedlings of *Solanum lycopersicum*, about four to six inches high, took the inoculation within two days (Plate VIII B). The photo was taken three days after the plant was inoculated. The seedlings soon wilted. No sporangia were produced on them but sections showed the presence of *Phytophthora* hyphæ. When leaves of older plants were inoculated they got blighted and blackened near about the point of inoculation ; the diseased area was always localised, even when growing points of big plants were inoculated. Fruits could not be inoculated, even when wounded.

The inoculation proved fatal to seedlings of *Solanum Melongena*, only when they were about four inches high and bore two or three small leaves. In this case also no sporangia were produced. The effect of the inoculation in the case of big mature leaves was localised.

Lilac plants did not take the inoculation vigorously. Inoculated leaves turned black. Sporangia were produced only when the diseased leaves were placed in water. The leaf buds did not get attacked when they were surrounded by a jacket of water containing motile zoospores. This method was used with success by Klebahn¹ in inoculating leaf buds of lilac with *Ph. Syringæ*.

¹ Klebahn, H. *loc. cit.*, p. 55.

Plants of two species of *Oenothera* took the inoculation quite readily in three days, both on leaves and stems. The inoculated area turned brown at first and then black. Both terminal and intercalary sporangia were produced on the surface.

Salpiglossis variabilis, *Gilia nivalis* and mixed spp., *Clarkia elegans*, *Schizanthus retusa* and mixed spp., and *Fagopyrum esculentum* became victims of the inoculation. Only young seedlings were experimented upon.

Opuntia Dillenii and two species of *Cereus* could not be inoculated even through wounds or on growing points. Cacao fruits and apples, even when wounded, did not take the inoculation. The fungus could not attack leaves and corms of *Colocasia antiquorum*, seedlings of *Lepidium sativum*, cuttings and plants of *Panax*, tobacco, *Cleome* sp. (even when incubated at 18°C.), four species of *Jasminum*, *Phaseolus lunatus* and *Phaseolus vulgaris*. Areca nuts, only when inoculated through wounds, produced a copious aerial mycelial growth in four days (Plate IX B). Sporangia were borne on long stalks, they were not sessile as in *Ph. omnivora*, var. *Arecae*.¹

In June, 1912, *Sesamum indicum* was sown in a field where castor was grown the previous year. Stray castor seeds fallen from the pickings of that year had germinated in June. About fifty per cent. of them were attacked by *Phytophthora*. When the seedlings of *Sesamum indicum* grew up amidst these surroundings they were found to be attacked by a *Phytophthora*. The leaves had blackened and got curled exactly like the leaves of potato attacked by *Ph. infestans*. The attack was not very severe, only a few seedlings being killed outright. It remained confined only to seedlings about eight to twelve inches in height. The sporangia resembled in size and shape those of the castor *Phytophthora*. The zoospores successfully inoculated castor seedlings, producing the same effect on them as if they were inoculated with *Ph. parasitica*. Seedlings of *Sesamum indicum* produced the characteristic leaf curl when inoculated

¹ Coleman, L. C. loc. cit., p. 51.

with *Ph. parasitica*. These successful cross inoculations show that the fungus is the same in both these cases.

The infection experiments show that some of the plants that are susceptible to infection by *Ph. omnivora*, *Ph. omnivora*, var. *Areceæ* and *Ph. Faberi* can be inoculated by the *Phytophthora* under investigation as well. Though seedlings of *Clarkia elegans*, *Gilia nivalis* and mixed spp. and *Schizanthus retusa* and mixed spp. died of the inoculation, no sporangia were found on them till they were placed in water for about a day. Sporangia are, however, produced on the surface of these seedlings when they are inoculated by any of the above three named species. Coleman found not only sporangia but oospores as well in the tissues of *Clarkia* inoculated with the areca and cacao *Phytophthoras*, so did de Bary in seedlings inoculated with *Ph. omnivora*. An important difference presented by the castor *Phytophthora* from *Ph. omnivora*,¹ *Ph. omnivora*, var. *Areceæ*,² *Ph. Faberi* and from *Ph. Syringæ*³ as well, lies in the former not inoculating *Cereus* even when wounded; while the latter four readily infect it and produce in its tissues oospores as well. *Lepidium sativum* and *Cleome* are susceptible to the attack of *Ph. omnivora*, but not to that of the castor *Phytophthora*. De Bary failed to inoculate *Solanum tuberosum* and *Lycopersicum* and Coleman succeeded in inoculating only their seedlings and those of *Solanum Melongena*. My results are identical with Coleman's. Even though the castor *Phytophthora* inoculated some of the hosts of *Ph. omnivora*, var. *Areceæ* and *Ph. Faberi*, still no relationship can be claimed for them with the former as it failed to inoculate wounded and unwounded fruits of cacao, and the cacao *Phytophthora* did not cross inoculate seedlings and older plants of *Ricinus*, while areca nuts could be inoculated only through wounds. Sporangia were not sessile as those of the areca *Phytophthora* but they were borne on long stalks.

¹ Klebahn, H. loc. cit., p. 69. Himmelbaur, W. Zur Kenntnis der *Phytophthora*, Aus dem Jahrbuch der Hamburgischen Wissenschaftlichen Anstalten, XXVIII, 1910, p. 41.

² Coleman, L. C. loc. cit., p. 178.

³ Klebahn, H. loc. cit. Himmelbaur, W. loc. cit.

Whatever affinity the inoculation experiments reveal between the fungus under study and *Ph. omnivora*, *Ph. omnivora*, var. *Arecae* and *Ph. Faberi*, the study of these fungi in pure cultures shows very little in common between the first and the last three. Hartig¹ found oospores of *Ph. Fagi* in water cultures. His results have been confirmed by de Bary² and recently by Klebahn,³ who studied *Ph. omnivora* from beech seedlings in pure cultures, and by Himmelbaur,⁴ whose careful study of *Ph. Fagi*, the cultures of which were supplied to him by Klebahn, and of *Ph. cactorum* have brought him to the conclusion that these two species cannot be merged into each other, under the common name of *Ph. omnivora*, as is done by de Bary. He also got the oospores of *Ph. cactorum*⁵ in water cultures. Though I have worked with water cultures of castor *Phytophthora* for months together I have never found the slightest trace of the formation of oospores in these cultures. Klebahn also succeeded in growing *Ph. omnivora* on sterilised potatoes and carrots, on which media not only sporangia were formed but apparently oospores as well. *Ph. Syringae* has also produced oospores on carrots and Coleman has found oospores of *Ph. Faberi* on sterilised potatoes. The castor *Phytophthora* thus disagrees with these fungi by not growing on sterilised carrots and by producing on sterilised potatoes like *Ph. omnivora*, var. *Arecae* only sterile mycelium and that too only when inoculations were made on the cut surface or on the wounded rind. *Ph. omnivora*, var. *Arecae* luxuriantly produced sporangia when grown on sterilised flies and a rich sterile mycelium on boiled rice. The castor *Phytophthora* thus differs from this fungus in growing very poorly on sterilised flies and producing sporangia and "resting" conidia on boiled rice.

The most important difference presented by the castor *Phytophthora* from these and all the other species of this genus, except *Ph.*

¹ Hartig, R. loc. cit., p. 45.

² De Bary, A. Untersuchungen über die Peronosporen und Saprolegnien. Beiträge zur Morph. und Physiol. der Pilze, 1881, p. 22.

³ Klebahn, H. loc. cit., p. 71.

⁴ Himmelbaur, W. loc. cit., p. 50.

⁵ Ibid., p. 54.

*Colocasia*¹, lies in the development of the sexual organs. In no other *Phytophthora* except *Ph. Colocasiae* has the oogonium been invariably found either to originate from within the antheridium or to arise from a separate stalk which penetrates the antheridium and grows through it. Clinton, indeed, is not certain that in *Ph. Phaseoli*² the oogonial hypha may not sometimes penetrate the antheridium. Judging from his microphotographs it seems that the oogonial stalk lies within the antheridium but his earlier drawings of these oospores are different from the microphotographs. Hartig³ has found in *Ph. Fagi*, oogonia in rare cases seated on antheridia and from his observations of these sexual organs, evidently mature, he supposes the base of an oogonium to have blown out into an antheridium. He says: "Ausnahmsweise und zwar vielleicht dann, wenn in nächster Nähe des intercellularen Oogoniums kein anderweites Mycel sich findet, von dem aus die Entwicklung des Antheridiums erfolgen kann, schwillt der Oognienträger unmittelbar unter dem Oogonium blasig an und wird direct zum Antheridium, wie Fig. 24b zeigt. So deute ich wenigstens die zuweilen auftretenden Stellungen des Oogoniums auf dem Antheridium selbst." The figure to which Hartig refers bears some resemblance to our figures; there seems therefore some probability that the oogonium which he found seated on an antheridium had arisen from within it. De Bary does not make any mention of the type of antheridium observed by Hartig. Klebahn who has recently studied *Ph. omnivora* (obtained from seedlings of beech), in pure cultures, also does not seem to have come across such an antheridium, for he makes no mention of it, nor does Himmelbaur in his more recent researches on *Ph. Fagi* and *Ph. cactorum* refer to it. But supposing what Hartig calls an hypogynous antheridium to be in reality an antheridium, through which the oogonium has originated, still the castor *Phytophthora* has other points of disagreement from this fungus, besides those already found from inoculation experiments and from the study of their growth on artificial cultures. Hartig,

¹ See footnote 6 on p. 189.

² Clinton, G. P. *loc. cit.*, 1907-1908 and 1905.

³ Hartig, R. *loc. cit.*, p. 49.

de Bary, Klebahn and Himmelbaur have found oospores being developed in the ordinary way, *viz.*, by the sexual organs arising either from two different branches or from two different stalks of the same branch and the antheridium meeting the oogonium to fertilise it by means of a fertilising tube, while the formation of a hypogynous antheridium was only occasionally met with by Hartig. On the other hand in our *Phytophthora* this customary mode of development of the sexual organs has never been found. Again the oospores are smaller than those of *Ph. omnivora*. De Bary does not give any measurements of oospores of *Ph. omnivora* but he says that they fill two-thirds or four-fifths of the oogonium, which ranges between 24 and 30 μ in diameter. Klebahn gives no measurements at all. Himmelbaur found the oospores of *Ph. Fagi* to be yellowish and ranging between 22-30 μ in diameter, and those of *Ph. castorum* brownish and measuring 30-40 μ in diameter. While the oogonia of the castor *Phytophthora* measure as a rule 18-25 μ , on an average 23.8 μ , if we exclude the secondary thickness formed on the walls of oogonia found in oat juice agar; and the oospores are hyaline and measure 15-20 μ in diameter, on an average 18.6 μ . The oospores of the castor *Phytophthora* being both lateral and intercalar would seem to offer a point of difference from *Ph. omnivora*, but much importance cannot be given to this; both Hartig¹ and Klebahn² have found them to be mostly terminal in *Ph. Fagi* while Himmelbaur³, who had the pure culture of this fungus from Klebahn, found them to be mostly intercalar.

From Coleman's figures it cannot be definitely determined whether or not the antheridium of *Ph. omnivora*, var. *Arecae* contains the oogonial stalk within it; from his description of the mode of fertilisation it is quite evident that it takes place by means of a fertilising tube, which has never been found in the *Phytophthora* on castor. Again the oospores of the former are much larger; they measure 26-30 μ in diameter.

¹ Hartig, R. *loc. cit.*, p. 49.

² Klebahn, H. *loc. cit.*, p. 72.

³ Himmelbaur, W. *loc. cit.*, p. 50.

Ph. Faberi not only shows points of disagreement in inoculation experiments and in its growth on artificial media but also in its oospores. Both von Faber and Coleman consider them to be parthogenetic, as they failed to find antheridia; but Rorer¹ has found them as his figure (Plate XVIII, Fig. 14), distinctly shows the presence of an antheridium. He has also found chlamydospores, which neither of the former investigators seem to have found. Perhaps as already stated they have mistaken these chlamydospores for parthogenetic oospores. According to von Faber the oospores measure 45μ in diameter, while Coleman says they are $22-45\mu$ in diameter. Rorer measures them between 38 and 40μ and the chlamydospores between 30 and 50μ . Whichever of these measurements are correct, they show that the oospores are much bigger than those of the castor *Phytophthora*.

Apart from the already noted differences that exist between *Ph. Syringæ* and our *Phytophthora* there are also those of sporangia and oospores. The sporangia of *Ph. Syringæ*, unlike those of the other species of the genus, have flat lid-like papillæ instead of raised semi-globular papillæ. The oospores are yellowish and much bigger. According to Himmelbaur they are 30μ in diameter and according to Klebahn $18-30 \times 17-25\mu$.

As we have seen *Ph. Phaseoli* perhaps offers some resemblance in the mode of the development of its oospores to the *Phytophthora* on castor. But a study of these two species leaves no doubt as to their being quite different from each other. The mycelium of the American fungus² is only intercellular; the hyphæ emerge singly or several in a cluster solely through stomata; the conidiphores are simple or, unlike its Indian ally, more commonly branched at the base; they measure $300-475\mu$ in length; while those of *Phytophthora* on castor measure, as a rule, $100-300\mu$. Again the former have on them half a dozen or more nodulose swellings which show the points of attachment of sporangia and the sporangia measure $17-35 \times 28-42\mu$. These swellings have never been found on the con-

¹ Rorer, J. B. loc. cit.

² Thaxter, R. A new American *Phytophthora*. Bot. Gaz. XIV, No. 11, 1889, p. 273.

diophores of the latter as they bear only single terminal sporangia which are bigger. The antheridia¹ differ from those of the castor *Phytophthora* in being temporary; the oogonia are much bigger, as they measure $23-38\mu$ in diameter and loosely envelope the oospores, which measure $26-28\mu$ in diameter and possess a thicker wall. In corn meal juice agar, Clinton² found practically no aerial growth but a slight embedded growth in which oospores were developed. But our fungus formed a fairly good aerial growth producing sporangia. Oospores were never developed in this medium. Again in corn-meal there was practically no growth, but on this medium the castor *Phytophthora* gave a copious aerial growth.

Ph. Thalictri, which Clinton³ suspects to be identical with *Ph. Phaseoli*, differs from our fungus in having larger and branched conidiophores, smaller sporangia and bigger and more thick-walled oospores, which do not differ materially from those of *Ph. Phaseoli*. The oogonia measure $25-33\mu$, are reddish brown and loosely envelope the oospores, which have $3-4\mu$ thick walls and are $18.5-25\mu$ in diameter.

Ph. infestans presents no point of agreement; the sporangio-phores are branched and nodulose, the sporangia are smaller ($27-30 \times 15-20\mu$) and the oogonia and oospores much bigger ($34-50\mu$ and $24-35\mu$ respectively).

There is absolutely no relationship between the *Phytophthora* on castor or *Ph. Colocasiæ* and *Ph. Nicotianæ*, as apart from morphological differences, the castor *Phytophthora* failed to inoculate tobacco seedlings and *Colocasia antiquorum* and the parasite of the latter did not cross-inoculate seedlings and plants of castor.

It is clear from all that has been said above that the *Phytophthora* on castor is quite different from the known species of this genus. I therefore propose to name it *Ph. parasitica* nov. spec.

¹ Clinton, G. P. *loc. cit.*, 1905, p. 293.

² *Ibid.*, *loc. cit.*, 1907-1908, p. 905.

³ *Ibid.*, p. 894.

PHYTOPHTHORA PARASITICA NOV. SPEC.

Maculis amphigenis, orbicularibus, centro areolatis, dilute umbrinus, anulatis, solitariis vel confluentibus; mycelio intermatriciali, ex hyphis inter-et intracellularibus, primo continuis tandem septatis, $3-9\mu$ crassis, constantibus; haustoriis sparsis, digitatis vel subglobosis, raro ramosis, sporangiophoris 100 to 300μ longis, sporangis terminalibus, interdum intercalaribus vel lateralibus, plerumque ovoideis, subinde globosis $25-50 \times 20-40\mu$; zoosporis $8-12 \times 5-8\mu$; sporis perdurantibus globosis, flavidis, $20-60\mu$, membrana crassa, levi; oogoniis et antheridiis in vitro cultis; oogoniis intercalaribus vel lateralibus, globosis, levibus vel rugosis, melleis, $18-25\mu$ ($23-8\mu$), pedicellis per antheridia penetrantibus: oosporis globosis, $15-20\mu$, ($18-6\mu$), membrana crassa, mellea, levi.

Hab. in foliis seminisque Ricini communis et Sesami indici, Indiæ orient.

Note.—While this paper was in the press *Sida cordifolia* from Dacca and *Sesamum indicum* from Saharanpur were reported to be attacked with *Phytophthora*.

In nature the parasite on *Sida cordifolia* has been found only on the flowers. Healthy flowers, leaves and stems of this host were inoculated with *Ph. parasitica*. The inoculation proved successful only in the case of flowers. It seems, therefore, not improbable that the species concerned in the Dacca specimens was *Ph. parasitica*.

The diseased leaves of *Sesamum indicum* have characteristic round spots which are either solitary or confluent. On the upper surface they have a brownish centre, surrounded by a whitish pale or dull green area which in turn has a brown areola. On the lower surface the brown centre is surrounded by a more dull green area which has an areola of a pale brown colour. Sporangia borne on long sporangiophores, which resemble those of *Ph. parasitica* on castor leaves, are found on the upper surface. They are slightly smaller than those found on castor leaves but they agree well in shape.

EXPLANATION OF THE PLATES.

PLATE I.

Upper and lower surfaces of a diseased castor leaf.

PLATE II.

- Fig. 1. Surface view of a castor leaf showing sporangiophores emerging through stomata and between the cell-walls of contiguous epidermal cells.—Somewhat diagrammatic. Drawn by Dr. E. J. Butler. X 320.
- " 2. Transverse section of the stem of a castor seedling showing the presence of hyphae in the vessels and in the bundle sheath. Drawn by Dr. E. J. Butler. X 320.
- " 3. Longitudinal section of a pitted vessel showing a hypha leaving through a pit. X 640.
- " 4. Transverse section of the woody portion of the pericarp of a fruit showing the hyphae boring through the lignified cell-walls by means of fine projection tubes. X 640.
- " 5. Transverse section made at the collar of a castor seedling showing palmately branched hyphae in cells of the cortex. X 640.
- " 6. Transverse section of a stem of a castor seedling showing hyphae swelling into globular heads just underneath the epidermis before breaking through it. X 405.
- " 7. Transverse section of a stem of a castor seedling. A cluster of hyphae breaking through the epidermis. On the left is the swollen head of a hypha just below the epidermis. X 405.

PLATE III.

- Fig. 1. A sporangium producing a highly branched vegetative mycelium when kept in the dark for twenty-four hours. (From a water culture.) X 100.
- " 2. Transverse section of a stem of a castor seedling. A is an intracellular branch of an intercellular hypha. At B are finger-shaped haustoria. X 405.
- " 3. Button shaped haustoria. X 405.
- " 4. A branched haustorium. X 405.
- " 5. Transverse section of a castor leaf. Two germ-tubes entering it through a stoma. X 405.

- Fig. 6. A germ-tube entering a castor leaf by breaking through the upper wall of its epidermal cell. X 405.
- " 7. A germ-tube entering a castor leaf between the walls of two contiguous epidermal cells. X 405.
- Figs. 8—11. Surface views of castor leaves showing the three ways in which the germ-tube enters the leaf. In Fig. 10 the germ-tube crosses over a stoma before entering the leaf. X 405.

PLATE IV.

- Fig. 1. Papilla swelling into a vesicle in which the sporangium is discharging mature zoospores. (Fixed with iodine.) X 405.
- " 2. An advanced stage of the same. (Fixed with iodine.) X 405.
- " 3. The vesicle has dissolved; the zoospores are still clumped together. (Fixed with iodine.) X 405.
- " 4. The papilla has dissolved but the zoospores are still within the sporangium. X 405.
- " 5. The discharge of zoospores in the normal way. The zoospore that is just out of the sporangium is linked to the one immediately behind it by a protoplasmic neck. X 640.
- " 6. Zoospores. A shows two zoospores linked together by a protoplasmic neck and possessing two cilia. X 640.
- " 7. A zoospore which came to rest at 10-20. X 640.
- Figs. 8—24. Successive stages in its germination. Fig. 8 drawn at 10-35. Figs. 9 to 14 drawn at intervals of five minutes. Figs. 15 to 24 drawn at 11-10, 11-45, 12, 12-35, 12-45, 1-20, 1-40, 2, 2-25 and 3-15 respectively. X 640.
- Fig. 25. Germination of zoospores by two germ-tubes. X 640.
- " 26. A motile mass of protoplasm with five visible cilia. (Freehand drawing.)
- " 27. The same in its resting stage. Drawn at 10-30. X 405.
- Figs. 28—34. Successive stages of its germination. Observe the gradual disappearance of the outer wall. These figures were drawn at 10-15, 10-55, 11-5, 11-25, 12-10, 1 and 3 respectively. X 405.
- Fig. 35. A sporangium germinating into a secondary sporangium. X 405.
- " 36. A bi-papillate sporangium. X 405.
- " 37. A sporangium producing in water cultures secondary and tertiary sporangia when kept under normal conditions for twenty-four hours. X 100.

PLATE V.

- Fig. 1. Typical aerial mycelium growing on French-bean juice agar and oat juice agar. (a) A "resting" conidium, (b) an intercalary sporangium. At (x) are cellulose deposits on the inner walls of the hypha. They have partially closed the hypha. The lowermost sporangium also shows a cellulose deposit on its inner wall and just above its point of attachment to the sporangiophore. X 405.

- Fig. 2. Hyphæ on the surface of the same two media.
- " 3. (a) Submerged hyphæ in the same two media. X 405. (b) Typical swollen budding hyphæ near about oospores in oat juice agar. X 405.
- " 4. Different kinds of septa. In (d) two cellulose plugs (x) grow in from the opposite walls which would have ultimately closed the hyphæ at this point, (e) is a bit of an old hyphæ growing in the substance of a medium showing septa at the origins of two branches. X 640.
- " 5. Cellulose plugs in hyphæ. In (a) x_1 completely closes the bore of the hyphæ. At x are three plugs which almost close it. In (b) x is an ingrowth which increases in size by caplike deposition of cellulose on it. In (c) are two ingrowths arising from the opposite walls and meeting each other thereby completely closing the hypha. In (d) x_1 is a thick cellulose plug. At x is a cellulose ingrowth of the inner wall of the hypha. X 640.
- " 6. Branches arising through septa. X 640.
- " 7. "Resting" conidium. X 405.
- " 8. "Resting" conidium germinating. X 405.
- " 9. "Resting" conidium. X 405.
- " 10. "Resting" conidium giving rise to secondary "resting" conidia. X 100.
- " 11. Immature intercalar "resting" conidium. X 405.
- " 12. "Resting" conidium germinating and producing a sporangium.
- " 13. Intercalar "resting" conidium. X 405.
- " 14. Abnormal sporangium germinating conidially; from potato juice agar. X 405.
- " 15. Germinating internal sporangium in the pericarp of a fruit. X 405.
- " 16. Portion of the mycelium from Peptone Broth. X 405.

PLATE VI.

- Fig. 1. Antheridium which is just being pierced by an oogonial hypha underneath it; observed vertically downwards. X 625.
- " 2. Oogonial hypha indenting an antheridium. (Freehand drawing).
- Figs. 3 and 4. Oogonial hypha originating within the antheridium. X 625.
- Fig. 5. Oogonial hypha that is lying within the antheridium after penetrating it. X 625.
- " 6. Same as above, the antheridium is intercalar. Optical section. X 625.
- " 7. Same as Fig. 5. X 625.
- Figs. 8—11. More advanced stages of Figs. 5-7. The oogonial hypha boring its way out of the antheridium. Figs. 8 and 9. X 625.
- Fig. 10. The oogonial hypha is on a lateral stalk. X 625.
- " 11. A mature intercalar oogonium. The septa in the antheridium have cellulose thickenings on them. X 1165.
- Figs. 12—16 and 17—22. Mature oospores. X 1165.



- Fig. 12. Oogonium originating within the intercalary antheridium. Optical section.
- " 13. Oogonium borne on a short stalk, not sessile on the antheridium.
- " 14. Hypha bearing the oogonial stalk within the antheridium swelling at its point of entrance.
- " 15. Oogonium borne on a lateral branch, the point of entrance of the oogonial hyphae is clearly visible. The hypha enters the antheridium from below and leaves it at its apex. The dotted lines indicate the hypha underneath the antheridium.
- " 16. Oogonium separated from antheridium which persists enclosing its stalk.
- " 17. Optical section of an oogonium originating from the base of an antheridium.
- " 18. Optical section of an oogonium developed from a separate oogonial stalk piercing the antheridium.
- Figs. 19 and 21. Optical sections, same as Fig. 17. In Fig. 19, there is a cellulose plug in the oogonial stalk.
- Fig. 20. Hypha bearing oogonial stalk penetrating the antheridium from above. The dotted lines show the stalk within the antheridium.
- " 22. Same as Fig. 15.

PLATE VII.

- Fig. 1. Oogonial thread (O) originating within the antheridium (A). X 500.
- " 2. Mature oospore from French-bean juice agar. Oogonium arising within the antheridium. X 400.
- " 3. Mature oospore from French-bean juice agar. Hypha bearing the oogonial stalk penetrating the antheridium on the left. X 400.
- " 4. A group of mature oospores from French-bean juice agar. X 400.
- " 5. A mature oospore from French-bean juice agar roughly manipulated. The passage made by the oogonial stalk within the antheridium is clearly visible. X 400.
- " 6. Oospore from oat juice agar. X 400.
- " 7. O. Oospores from French-bean juice agar.
R. "Resting" conidia. X 400.
- " 8. "Resting" conidia. X 400.

PLATE VIII.

- Fig. A. Seedling of Potato. The central shoot marked with an arrowhead was inoculated. The other two shoots served as controls. Photo taken five days after inoculation.
- " B. Seedlings of tomato. On the left is the inoculated seedling; the other was kept uninoculated as a check. Photo taken three days after inoculation.

PLATE IX.

- Fig. A. A pot containing a tobacco seedling on the left and a castor seedling on the right. Both were inoculated at the same time. Photo taken two days after inoculation.
- „ B. An areca nut inoculated through a wound. Photo taken six days after inoculation.

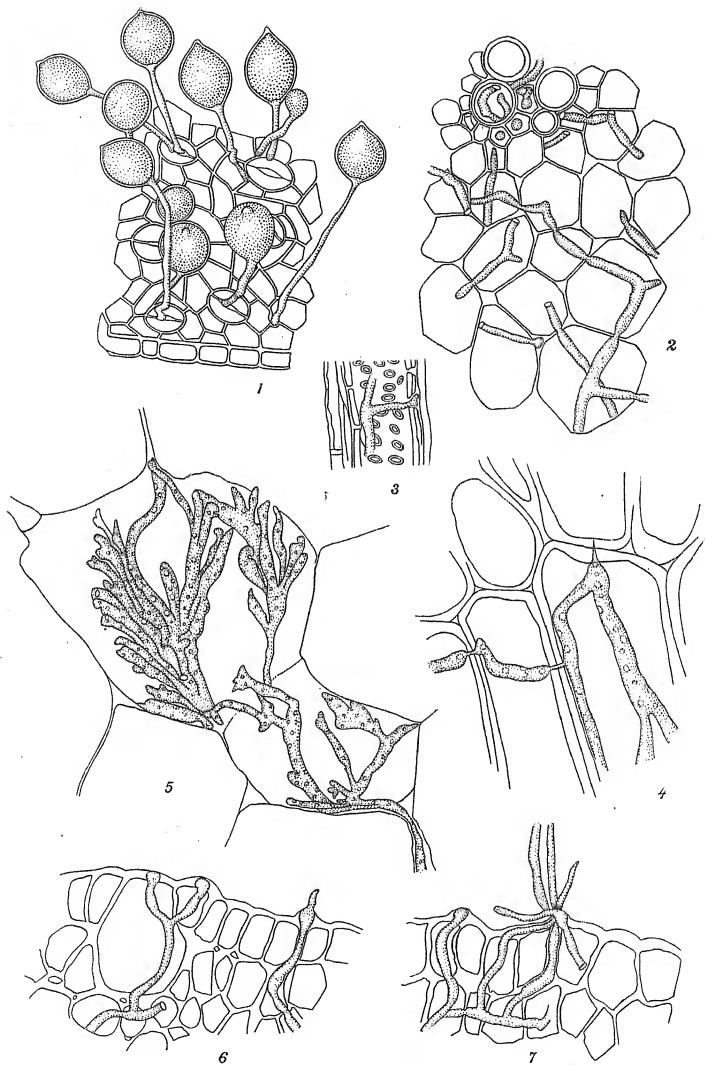
PLATE X.

- A castor seedling inoculated by surrounding it in a jacket of water containing motile zoospores. Photo taken three days after inoculation.

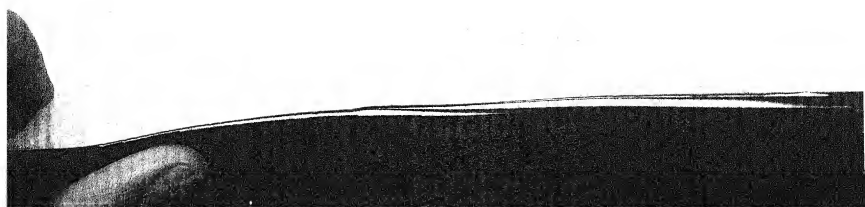


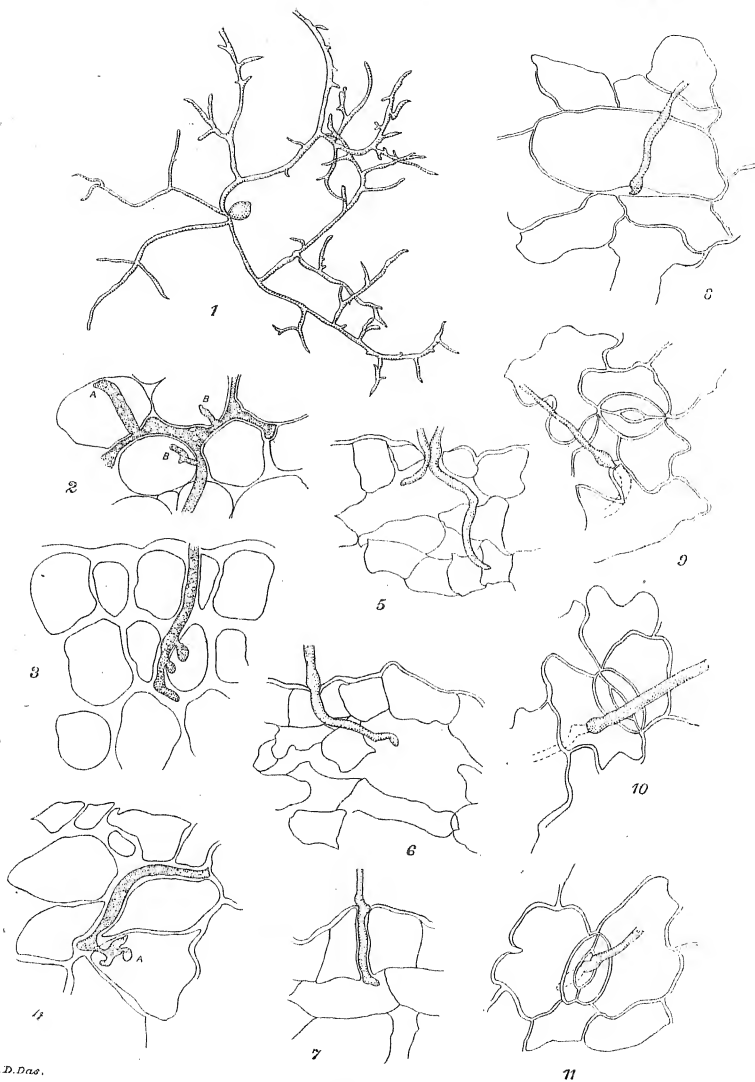
RICINUS LEAVES ATTACKED BY PHYTOPHTHORA PARASITICA.





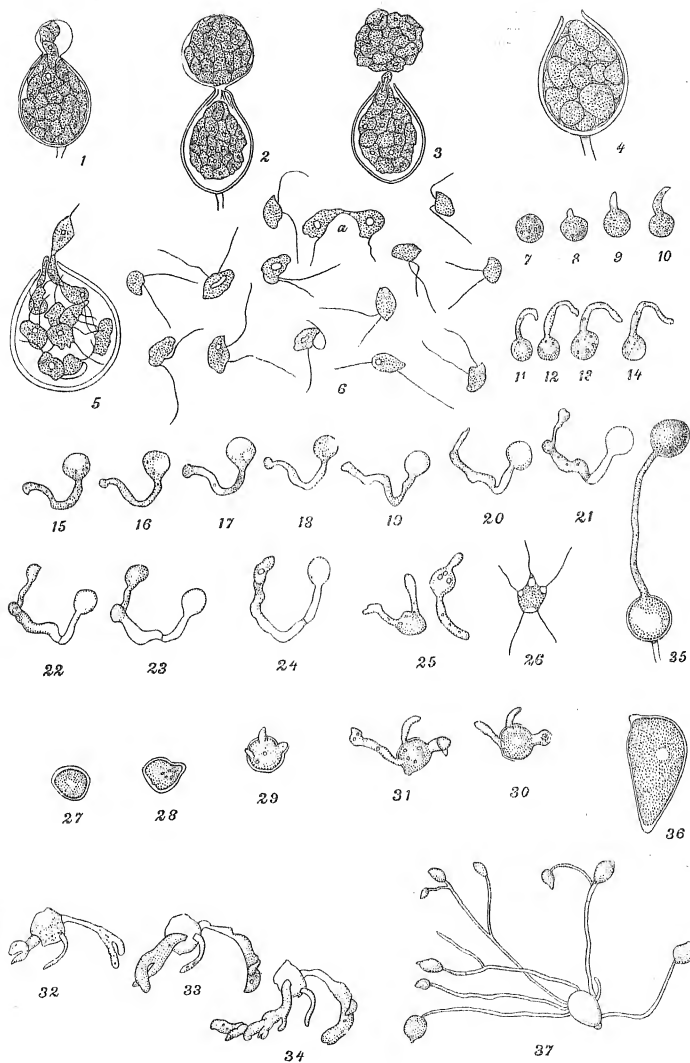
Phytophthora parasitica.





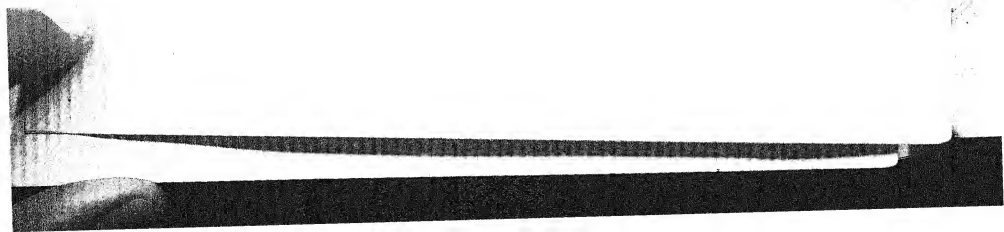
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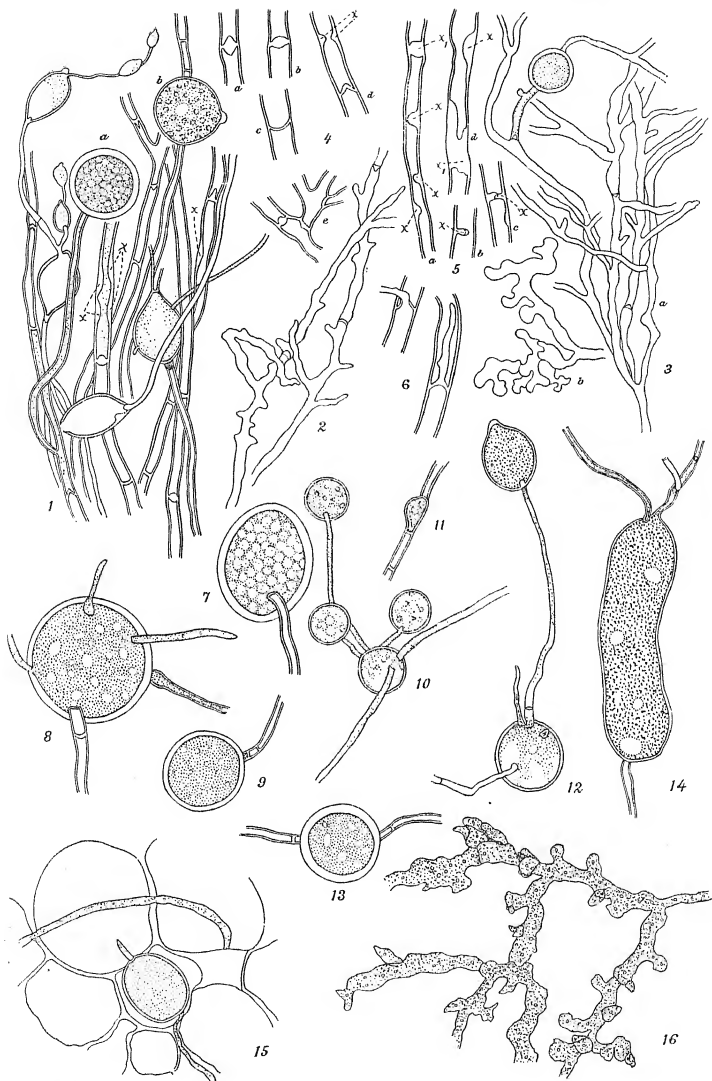
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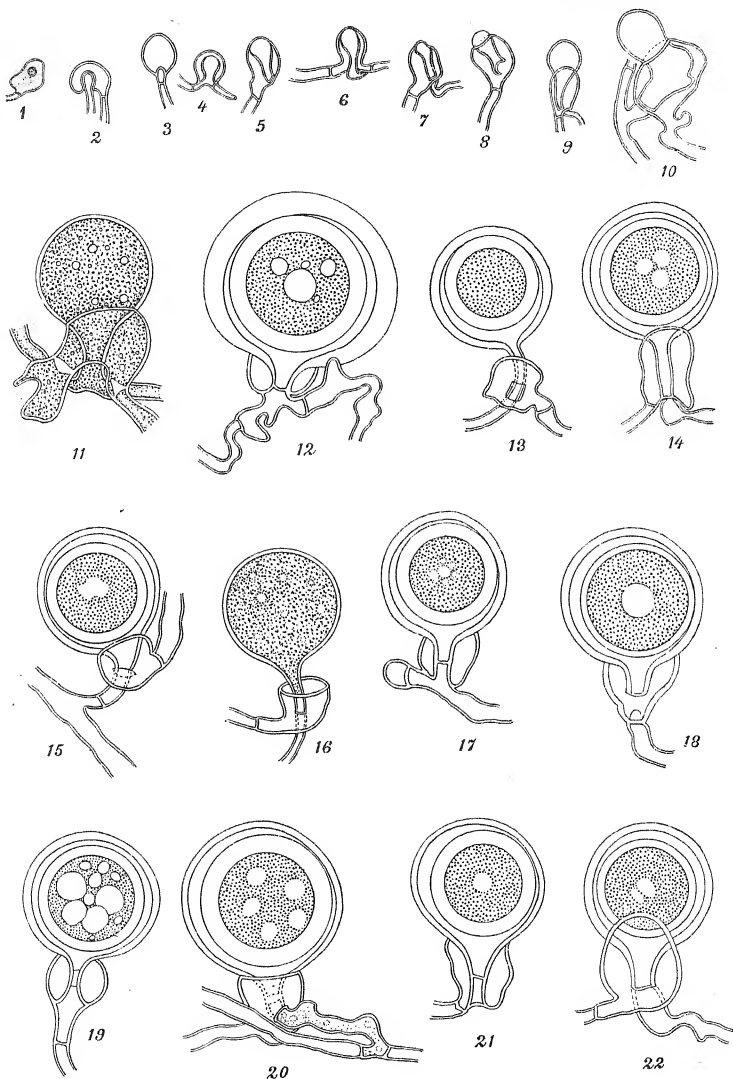
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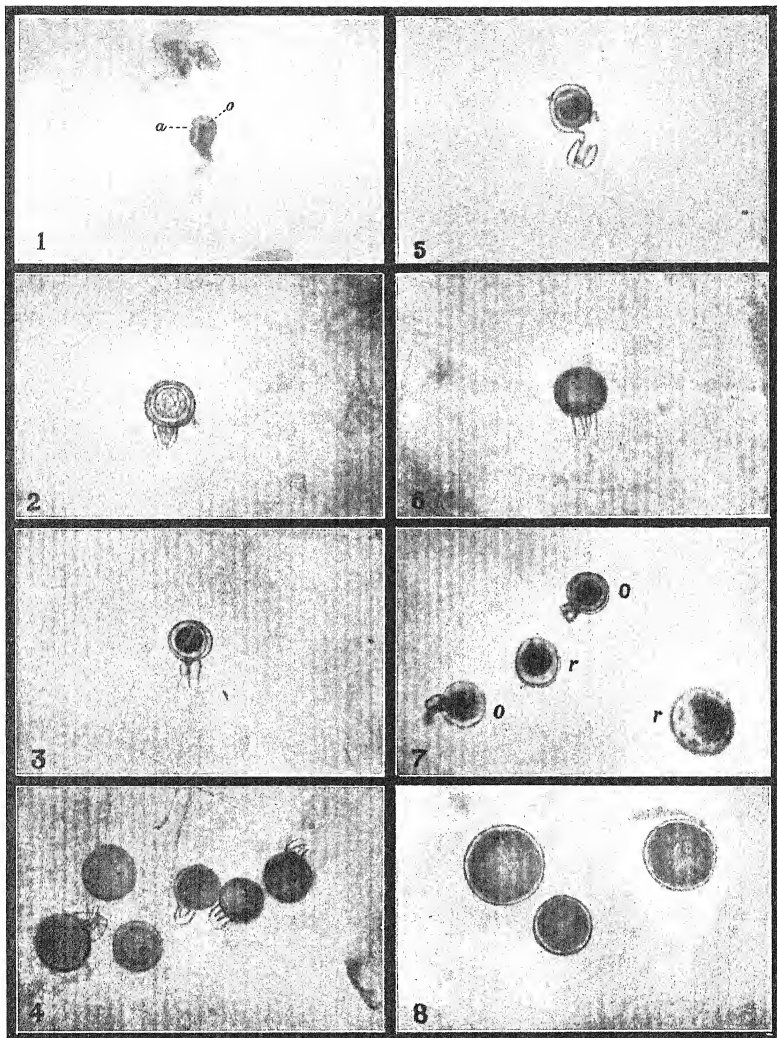
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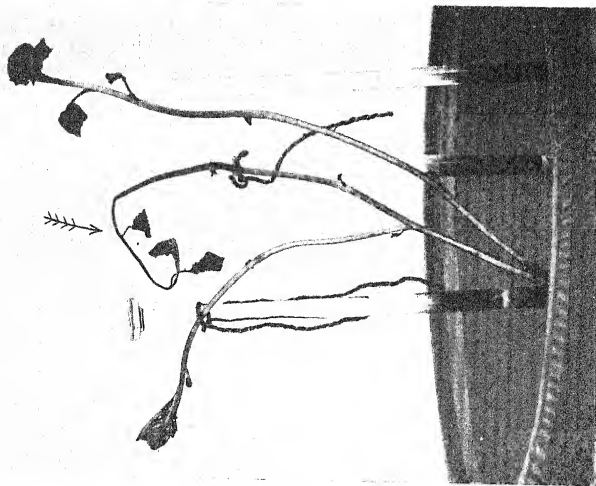


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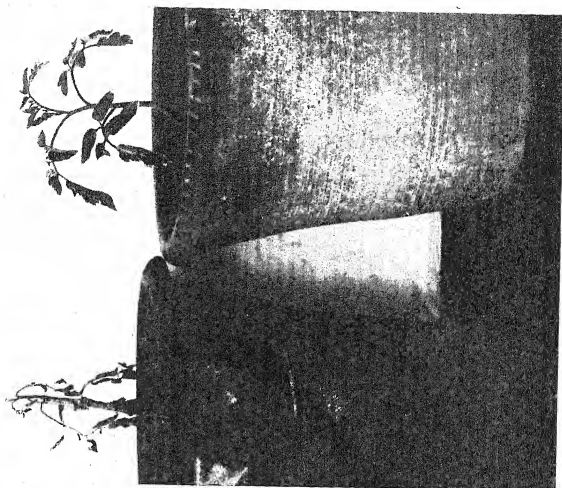
Phytophthora parasitica.



Phytophthora parasitica.



A

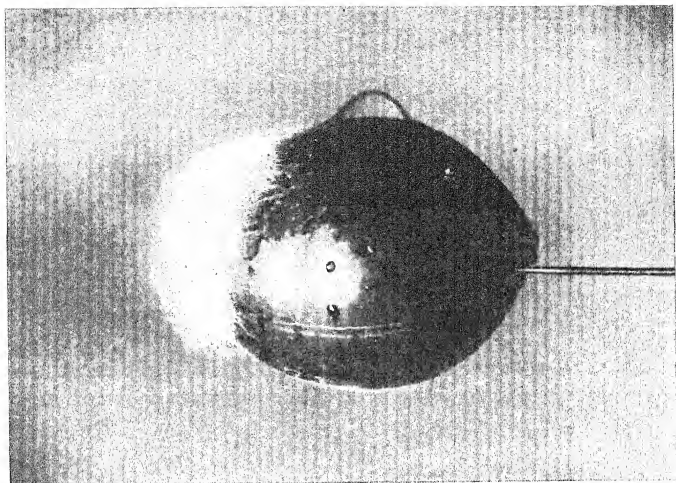


B

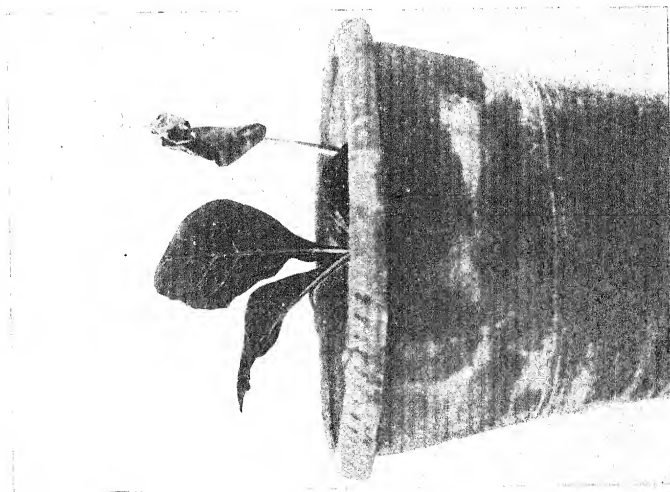
Phytophthora parasitica,
Inoculations on potato (A) and tomato (B).



PLATE IX.

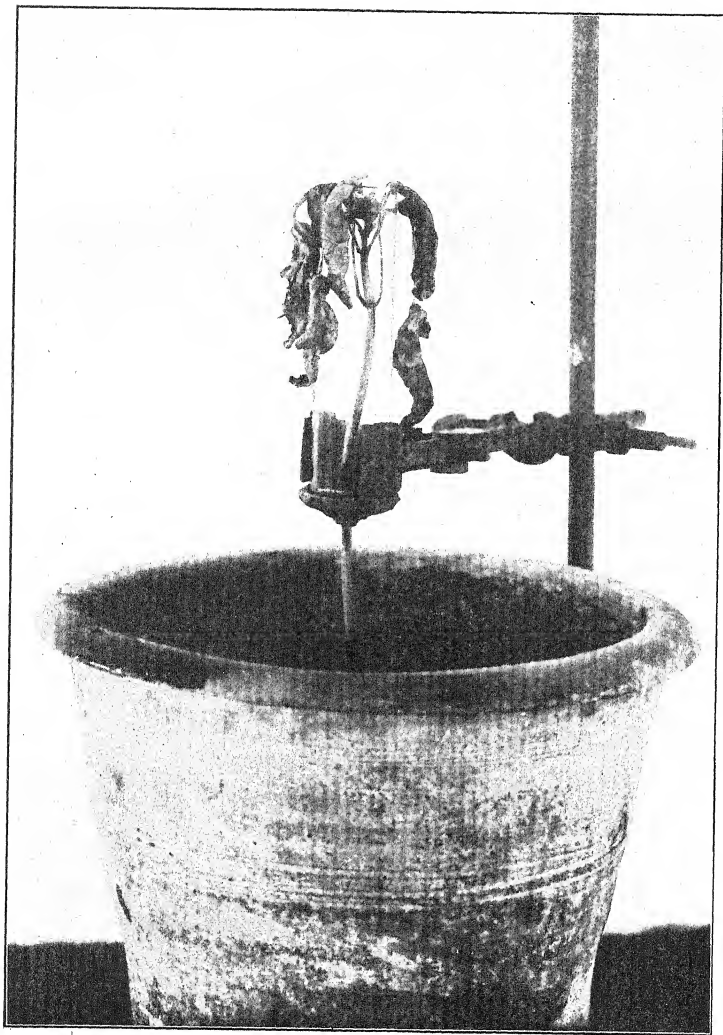


B

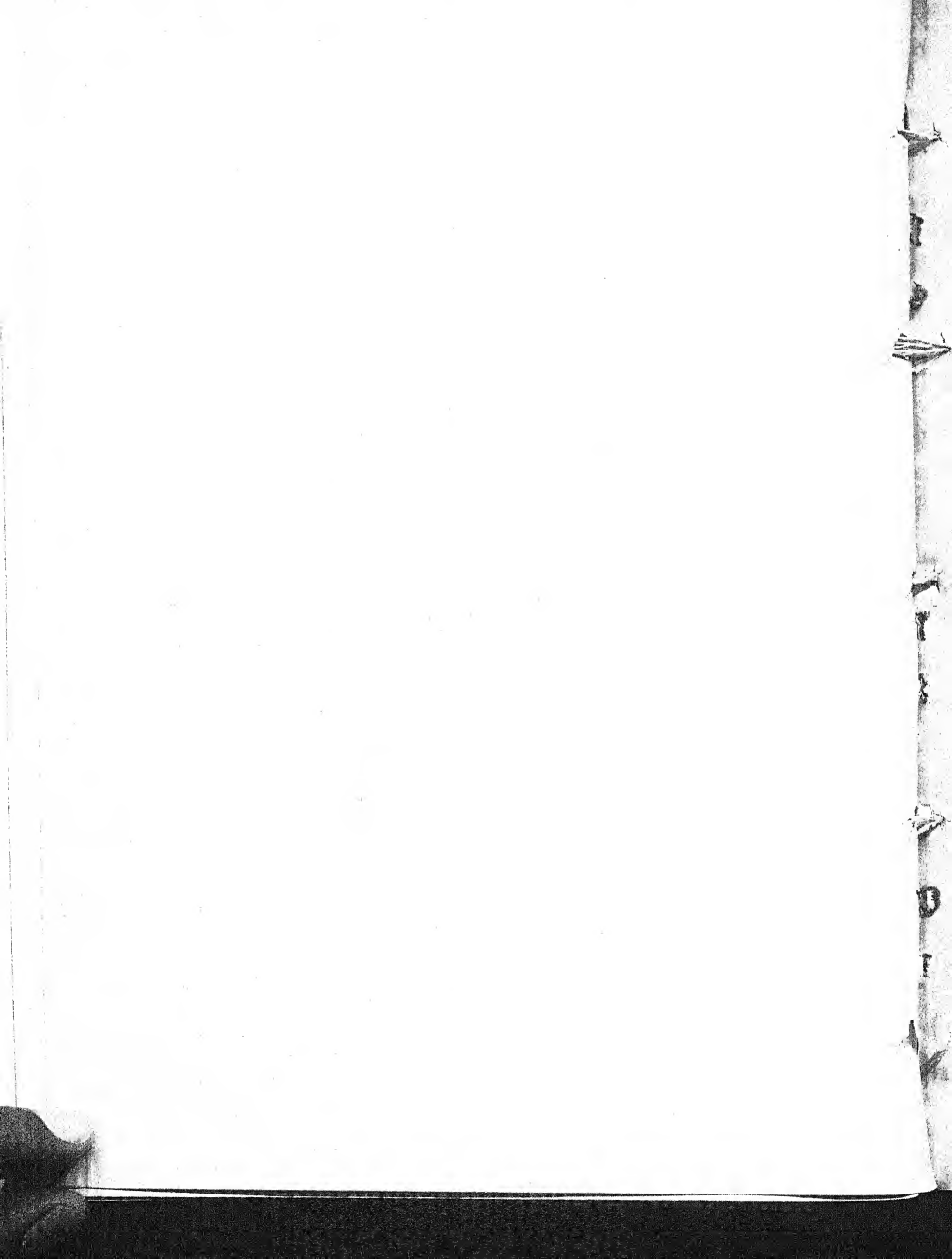


A

Phytophthora parasitica.
Inoculations on tobacco and castor (A) and arecanut (B).



Castor seedling inoculated with *Phytophthora parasitica*.



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BY

E. J. BUTLER, M.B., F.L.S.

Imperial Mycologist

BY

G. S. KULKARNI, L.A.G.

Mycological Assistant, Bombay Department of Agriculture



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COLOCASIAE BLIGHT
CAUSED BY PHYTOPHTHORA COLOCASIAE RAC.

BY

E. J. BUTLER, M.B., F.L.S.,
Imperial Mycologist,

AND

G. S. KULKARNI, L.Ag.,
Mycological Assistant, Bombay Department of Agriculture.



I. DESCRIPTION OF THE DISEASE.

Phytophthora Colocasiae Rac. was first described by Raciborski¹ in 1900. In Java, according to this author, it is widely distributed as a parasite on the leaves of *Colocasia esculenta* (= *C. antiquorum* Schott.) but does not cause much damage to the plant. His description is brief and practically confined to the naked eye appearance of the infected leaf and the sporangial stage of the fungus. It was not, apparently, obtained elsewhere than on the leaves of the host and was not cultivated.

The parasite does not appear to have been reported outside Java until 1907, when it was recorded from India, in collections made in 1905². More recently Sawada has encountered it in Formosa³; his description is in Japanese but illustrated by two plates, these being, so far as we know, the only published figures of the fungus. Sawada, on what appear to us to be entirely insufficient

¹ Raciborski, M. Parasitische Algen und Pilze Javas, I, 1900, p. 9.

² Sydow, H. & P., and Butler, E. J. Fungi India Orientalis, II. Ann., Mycol. V, 1907, p. 512.

³ Sawada, K. Infection of Taro. Rep. from Special Reports of the Formosan Agric. Exper. Station, II, 1911 (Japanese). We are indebted to Mr. S. Kamisaka of Bombay for a translation of this paper.

grounds, transferred the species to the genus *Kawakamia*, a genus founded by K. Miyabe for a fungus parasitic on the stems of *Cyperus tegetiformis*¹, and itself doubtfully tenable.

In India the distribution is wide, from Travancore on the south to Dehra Dun and Rangoon on the north and east, and in Bengal it is one of the commonest of the *Peronosporaceae*. It is found everywhere in the plots of *kachu* (*Colocasia antiquorum* Schott.) seen towards the middle of the rains in most villages. It also occurs in the wild specimens of the same plant common in moist localities. Raciborski's statement that it causes little damage is too sweeping. Sometimes, in favourable weather for its development, the leaves are so rotted that the entire plant is killed. In 1909, during a period of almost continuous cloud and rain in August, a number of plants died from the disease at Pusa and the growth of the rest was brought to a standstill. Further, the parasite commonly reaches the corn and sets up a dry rot during storage. This stage was not known to Raciborski but is probably more destructive, as a rule, than the leaf attack.

The disease first manifests itself ordinarily on the leaf. Small dark roundish specks appear which widen rather rapidly by centrifugal growth. The growth may be equal all round, in which case the spot remains circular, or may be more rapid in one direction than another, when we find oval, elongated or irregular patches (Pl. I). The main veins of the leaf often check extension for a short time but are soon crossed. Two or more spots may unite and ultimately a very large part of the leaf surface may be involved. In the early stages drops of a clear yellow liquid ooze out from the surface of the spots; later, the central portion assumes a yellowish-brown colour, dries up and may become perforated. The margins are often beautifully zoned, in different shades of brown, green and yellow; towards the periphery these zones reveal on careful examination a delicate white haze which is caused by the sporangial stage of the fungus. This external development of the parasite is,

¹ Miyabe, K., in Kawakami, T. "Kawakamia, Miyabe, a new genus belonging to *Peronosporaceae*." Tokyo, Shokwabo & Co., 1904.

however, much less prominent than in, for instance, the allied potato blight, and is sometimes difficult to detect by the naked eye. As the spot widens sporangia continue to be produced at progressively increasing distances from the centre.

The above may, in mild cases, constitute the whole extent of the attack. Frequently, however, the petiole becomes infected. The course of the disease here is similar to that on the leaf-blade except that the spots soon become elongated in the direction of the axis of the stalk. The damage caused is, however, greater, for if the spot is of any considerable size, it leads to such shrinking and disorganisation of the tissues that collapse of the structure above is brought about. The leaf hangs on the stalk and rapidly decomposes. Sometimes the petiole breaks at the point of worst attack, the part below drying up so rapidly as to prevent further progress. If this does not happen extension down the petiole may continue as far as its sheathing base; infection of the stem at this point was naturally looked for and though no definite case was seen, the ready extension down the petiole renders it not improbable. In *Phytophthora infestans* it was at one time believed that infection of the potato tubers, which is well known to occur, results from extension of the mycelium from the leaves and stalks down within the tissues. It is now more generally held to be due to direct infection from sporangia and zoospores fallen from the above-ground parts and germinating in contact with the tubers. Similarly, in *Colocasia* direct infection of the corm must occur and the more readily that it is not uncommon to find corms partly exposed at the surface of the soil. Though internal infection through the stem may occur, the fact that no actual case could be found suggests that external infection, from reproductive bodies formed on the leaves and petioles, is the more usual course.

In a few cases infection of the inflorescence was observed.

In a severe attack all the leaves may be lost and the plant killed. In milder cases the young leaves may be free from disease or only slightly involved, while the older ones are covered with patches of varying extent or are hanging rotted on their stalks,

The corms may be entirely lacking or, if formed, may be small and shrunken. Where only isolated spots occur there is little apparent injury to the plant and the corn matures normally. This is the condition which has been most frequently found. Nothing is clearer than the close connection between the intensity of the disease and the atmospheric conditions prevalent during the growth period of the host. Much the worst attack noticed at Pusa during the last six years occurred in 1909, a year when the rainfall was nearly double the normal, there was almost continuous cloud or rain during August and September, the total precipitation for these two months was 28.44 inches and the 8 A.M. relative humidity exceeded 80 per cent. on fifty-three out of sixty-one days. In the present year (1912) the disease had not appeared up to the end of September, the rainfall during the previous two months having been only 12.96 inches and the 8 A.M. relative humidity exceeding 80 per cent. on forty days only. Sawada notes that the disease is worse in shady than in sunny localities in Formosa.

Infection of the corm was first noticed in 1909. It has been observed each year since, though not to the same degree. The corm rot is sometimes apparent even in the field but more usually develops subsequently in storage. As in the tuber rot caused by *Phytophthora infestans* it appears to be, when not complicated by secondary organisms, a dry rot, the tissues being dry but rather soft to the touch. Wet rot may develop in stored corms in which *Phytophthora Colocasiae* can be detected but this is probably due to secondary invasion by various soil organisms.

II. MICROSCOPIC CHARACTERS OF THE DISEASE AND OF ITS CAUSE.

The mycelium of the parasite is present in all the parts which show external discoloration. It has been found in the leaf (including the petiole), the inflorescence and the corn. The hyphae are large, usually 5 or 6 μ , but varying from 4 to 9 μ in diameter, unseptate and branch copiously. In the leaf-mesophyll they are purely intercellular but epidermal cells are often traversed by hyphae both entering and emerging from the leaf. Haustoria

(Pl. II, Fig. 2) are sent freely into the cells of the pallisade and spongy parenchyma and sometimes into those of the epidermis. They are simple, or occasionally branched, finger-shaped processes, not differing from those of *Sclerospora* or *Pythium palmivorum* in any essential. In the petiole the hyphæ are still inter-cellular, being found especially in the air-spaces between the cells, and the haustoria are numerous and very distinct. Neither in the leaf nor in the petiole are the bundles ever penetrated. In the corm, on the other hand, not alone are hyphæ found in the storage cells, the mycelium being rather intra- than inter-cellular, but the bundles are commonly penetrated (Pl. II, Fig. 5). Haustoria-like branches extend from the inter-cellular hyphæ into the storage cells but they are often not clearly distinguishable from the ordinary branches of the mycelium, which themselves may extend from cell to cell without appearing to experience the slightest difficulty in penetrating the cell walls. This difference in habit according to the tissue attacked is of considerable interest and appears to suggest that it is not so much the structure of the wall, as the nature of the substances occurring within the cells, that determines whether penetration will take place or not.

In assimilating cells the chloroplasts commence to lose colour soon after infection takes place. Their sharpness of outline then gradually disappears and the individual plastids run together into a grumous mass in which, for a while, the starch granules are still visible. Then the cells turn brown, the phloem elements sharing in this change although not penetrated by the parasite. In the corm, as soon as rotting is well established, and in the centre of the leaf spots, starch is entirely absent from infected cells.

No trace of reproductive organs has been detected in any part of the internal mycelium. Sporangia are produced externally on short stalks, arising from the epidermis at the margins of the spots on both surfaces of the leaf blade and on the petiole. They come out in successive crops as the centrifugal spread of the fungus continues. They have not been observed occurring naturally on the surface of rotting corms but have been obtained by removing with

a sterile knife a slab from the margin of a rotting patch and dropping it into pure water in a sterile flask. In some cases a copious external growth was obtained by this treatment and mature sporangia were formed on the third day. The fungus maintains its vitality for a considerable time in the corm. Pl. III, Fig. 14 shows a hypha obtained by incubating a corm about a month after it was harvested and some three months after the disease had ceased to be active. It is probable too that corms kept under conditions which permit of moisture to accumulate, develop aerial hyphae in store and infect their neighbours. There is no reason to doubt that the parasite can persist from one season to another in the corms and, as in the allied potato blight, infection of the new crop possibly comes in part from these diseased tubers. Resting spores, whether sexually produced oospores or the thick walled reproductive bodies known as chlamydospores, have not been found in nature, though they have been obtained in artificial cultures.

On the leaf the sporangia are borne on short stalks as shown in Pl. II, Fig. 1. They are terminal and single but sometimes the stalk continues to grow by a branch arising below the sporangium, the latter being pushed to one side and a new sporangium being formed at the tip of the branch (Pl. III, Fig. 15). The sporangiophore narrows towards the apex to an extremely slender thread, sometimes not exceeding 1.5μ in diameter, and the sporangium falls with a part of this narrow stalk attached to its base. The fruitifying hyphae emerge, as shown in the figure, not only through stomata (the usual mode of emergence in *Peronosporaceae*) but also between or through the epidermal cells. They rarely exceed 50μ in length and, excepting in the rather infrequent case of more than one sporangium being borne, they are unbranched.

The sporangia are colourless, elliptical or pear-shaped, sometimes curved and less regular in shape than is the case with most species of *Phytophthora*. They are often very large, measuring when formed on the leaf from 38 to 60 by 18 to 26μ in diameter but varying within wider limits in culture. Sawada has observed sporangia up to 114μ in length on the leaf, but in India they rarely

exceed 60μ . The wall is thin and smooth and at the free end there is a broad blunt papilla. When mature each sporangium has a single large vacuole lying near the centre, the rest of the cell being filled with dense granular protoplasm (Pl. IV, Figs. 1 and 2). Germination may occur within half an hour after sowing in water, up to 20 large zoospores being liberated. The zoospores are more or less bean-shaped, one of the longer sides being convex and the other concave or plane (Pl. IV, Fig. 3). Each contains a small pulsating vacuole and two cilia arise near together from the concave or plane side, one projecting in front and the other behind while swimming. After swimming for some time they come to rest, round off, lose the cilia and become surrounded by a cellulose wall. At this period they measure from 10 to 13μ in diameter (Raciborski gives the measurement of the swimming spores as 15 to 18 by 9 to 12μ ; Sawada 14 to 18 by 9 to 13μ). Germination then occurs by a germ-tube, which may be protruded within half an hour after the zoospore comes to rest or within an hour after the liberation of the zoospore from the sporangium. The germ-tube grows and branches for a time (Pl. III, Fig. 10) but in water dies without forming any definite mycelium. Raciborski¹ describes a peculiar type of germination in this fungus. The zoospore, after coming to rest, puts out a short tube which swells at the tip to form a sort of secondary sporangium which may continue growth by a hypha or may open to liberate one or two secondary zoospores. This recalls the germination of the zoospores of *Pythium diacarpum*² and is to be considered as the physiological equivalent of the process of diplanetism in the *Saprolegniaceae*. Diplanetism is probably, as suggested by Hartog³, an adaptation to secure wider dissemination than could be obtained by the swimming spore alone. During the period of rest between the two stages of activity the spore can be passively carried to a distance by currents of water and can then reach a suitable substratum for further development

¹ *loc. cit.*, p. 10.

² Butler, E. J. An account of the genus *Pythium* and some *Chytridiaceae*. Mem. Dept. of Agric. in India, Bot. Ser., I, No. 5, 1907, p. 81.

³ Hartog, M. On the Formation and Liberation of the Zoospores in the *Saprolegnia*, Quart. Journ. of Microscop. Science, N. S., XXVII, 1887, p. 437.

during the second active stage. In some genera of *Chytridiaceae* the same purpose is apparently served by a period of quiescence of the spore, during which the cilia are retracted to be again protruded after a time sufficient to allow of a certain amount of passive dissemination¹. Sawada has also observed the formation of a secondary spore germinating by a hypha on the germ-tube of the primary spore, but the formation of secondary zoospores has not been observed either by him or by us and is certainly of infrequent occurrence.

Sometimes the zoospores fail to escape, one or more being left in the sporangium. After a time these cease to move, secrete a wall and germinate in the usual manner. The germ-tube appears to be unable to pierce the sporangial wall and can only emerge through the opening (Pl. III, Fig. 11).

Recently matured sporangia germinate for the most part by giving zoospores, when placed in fresh water. Frequently, however, a certain number fail to form zoospores but germinate directly by a hypha. In sporangia, from their natural habitat on the leaf, the germ-tube arises from the papilla, giving a stout branching hypha, which may form a mycelium or give secondary sporangia after a short growth. This type of germination will be more fully described (from cultures) below.

Some observations were made on the formation and discharge of the zoospores. The ripe sporangium contains a single vacuole of variable size. This is at first irregular and changes shape with the slow movements of the intersporangial protoplasm; then it becomes spherical and ultimately disappears suddenly. The protoplasm itself is at first coarsely granular and with a tendency to form clumps. Later on it becomes more finely granular and after the discharge of the vacuole it is almost homogeneous. About five minutes after the vacuole disappears, the first cleavage lines of the spore-origins become visible and the protoplasm contracts slightly so as to leave a clear space just inside the wall. Soon after, discharge occurs, in the manner so often described for *Phytoph-*

¹ Butler, E. J. *loc. cit.*, p. 121 and, Barrett, J. T. Development and Sexuality of some species of *Olpidiopsis*, *Ann. of Bot.*, XXVI, 1912, p. 215.

thora, the spores being fully demarcated and provided with cilia before they emerge to the outside (Pl. IV, Fig. 3). Often two or more zoospores remain attached, in extreme cases forming a mass in which the individual spores cannot be distinguished. At least ten spores must have been included in some of the masses expelled from feeble or contaminated cultures. These masses are provided with several or numerous cilia but always appear to break down and disintegrate without secreting a wall.

The influence of temperature on zoospore formation is marked. A portion of a culture was divided into two parts, one of which was placed in tap water in the cool incubator at 25°C., the other kept in the laboratory at 32°C., both being equally illuminated. Examined after an hour, the former was found to be discharging freely, the latter not at all. The higher temperature, however, merely inhibits zoospore production, without affecting the power to produce them when suitably cooled, as part of a culture which had remained in water for two hours at 30°C., without any but a very few sporangia having discharged, gave very numerous zoospores when placed in the cool incubator.

Light does not appear to influence the formation of sporangia when temperature conditions are suitable. A culture kept in the dark at 25°C. for a month from the date of sowing was found as fully provided with sporangia as any of the cultures in the laboratory. This differs from the results got by Dastur¹ with *Ph. parasitica*, where cultures kept in the dark remained sterile. No observations were made on the effect of light on the emission of zoospores but Coleman and Dastur have found that a certain amount of light is necessary for this process in *Ph. omnivora* var. *Areca* and *Ph. parasitica* respectively.

III. CULTURES OF PHYTOPHTHORA COLOCASIAE².

In recent years several species of *Phytophthora* have been successfully cultivated on artificial media. In the present

¹ Dastur, J. F. On *Phytophthora parasitica* nov. spec., a new disease of the Castor oil plant. Mem. Dept. of Agric. in India, Bot. Ser. V, No. 4, 1913.

² We are indebted to Mr. J. F. Dastur, First Assistant in the Mycological Section, Pusa, for much assistance in obtaining cultures of the fungus.

species considerable difficulty was experienced, but successful cultures were eventually obtained in the following manner. Healthy living corms were placed in contact with fructifying spots on the leaves, the surface of the corm being sliced off at the point of contact. By keeping the surrounding atmosphere saturated, aerial hyphæ developed on the leaf and penetrated the corm. The latter was then removed to a moist chamber and a good aerial growth of the fungus, bearing sporangia, developed after a few days. A healthy living corm was next sterilised by washing well in spirit and flaming off the excess of alcohol. It was then divided with a sterile knife and the cut surface placed so as to touch the aerial hyphæ of the previously infected corm. After 24 hours the sterilised corm was removed to a sterile moist chamber and gave in subsequent days a copious woolly growth. From this pure cultures were obtained by inoculating tubes of French-bean agar. Other tubes of this medium, as well as of corn meal, were inoculated directly from the first infected corms and purified from the bacteria which contaminated the first tubes by successive subculturing. Ultimately pure cultures were grown on the following media :—French-bean agar, corn meal, oat-juice agar, prune-juice agar, and glucose-peptone agar¹. Cultures on sterilised slabs of Colocasia corm (sterilised by intermittent steaming in the Koch and placed respectively in potato tubes with distilled water, and on the surface of

¹ These media were prepared as follows :—

French-bean agar.—50 grammes dried beans are pounded in a mortar, made up with about 300 c.c. distilled water, boiled for half an hour and strained through fine wire gauze. To this is added 10 grammes agar dissolved in a small quantity of water and the whole is made up to 500 c.c. with distilled water. After heating to mix thoroughly it is strained through fine cloth into tubes and autoclaved. The reaction was +2 of Fuller's scale.

Corn meal.—Maize meal moistened with distilled water.

Oat-juice agar.—50 grammes crushed oats are cooked with about 300 c.c. distilled water for an hour in the Koch and strained through wire gauze. To this is added 10 grammes agar dissolved in water enough to bring the whole to 500 c.c. After heating to mix it is strained into test-tubes and autoclaved. The reaction was +2 of Fuller's scale.

Prune-juice agar.—5 prunes boiled in 100 c.c. distilled water for 5 minutes and filtered. To this is added 7.5 grammes agar dissolved in a small quantity of water and, after thorough mixing, the whole made up to 500 c.c. with distilled water, run into tubes and autoclaved.

Glucose-peptone agar.—Glucose 10 grammes, meat extract 2 grammes, peptone 5 grammes, sodium chloride 2.5 grammes, agar 7.5 grammes, water 500 c.c.

moist corn meal and glucose-peptone agar media) failed to grow.

The best cultures were obtained on French-bean agar, corn meal and oat-juice agar¹. On corn meal the growth was dense and woolly and extended over the whole surface of the medium fairly rapidly. On French-bean agar the mycelium spread less rapidly and was not as dense as the last. On oat-juice agar there was less aerial growth than the others. Prune-juice agar and glucose-peptone agar gave poor thin growths. It is somewhat surprising that steamed Colocasia corm should, in spite of repeated inoculations, have failed to show any growth. In all the successful cultures sporangia were developed, provided the temperature was not too high. Oospores were much less common and were only formed in French-bean agar, buried in the medium, generally several near together at about one-eighth of an inch below the surface.

The sexual stage of several *Phytophthoras* is rarely developed. In the well-known case of potato blight, *Ph. infestans*, it has only recently been discovered (in the United States), in spite of continual searches during the past thirty years. It has never been encountered under natural conditions (the bodies described by Worthington Smith many years ago have not been accepted by mycologists generally as belonging to this fungus) but has been produced in artificial cultures only². The same is the case with the present fungus, as neither in Java, nor in Formosa, nor in India have oospores been found in or on the diseased plants. It is probable that in these two cases the absence of a resting-spore condition is to be correlated with the presence of a mycelium in the tubers, capable of passing over from one season to another.

The characters of the fungus in culture may now be described, little difference, except in the vigour of growth and the presence or

¹ cf. Clinton, G. P. Artificial cultures of *Phytophthora*. Conn. Exper. Sta. Report, 1907-08, p. 898, and Oospores of Potato blight, *ib.*, Report 1909-10, p. 760.

² Clinton, G. P. Oospores of Potato blight. Conn. Exper. Sta. Report, 1909-10, p. 753.

absence of oospores, being noticed on the three more satisfactory media, mentioned above.

The mycelium covers the surface of the medium rather slowly and forms a white aerial growth, on which are found mature sporangia in about a week or ten days at temperatures of 15 to 20°C. The hyphæ of the aerial mycelium are long, sparingly branched, about 4 to 7 μ in breadth and not varying abruptly in diameter (Pl. III, Fig. 4). Septa are frequent, but instead of being formed, as in the higher fungi, regularly from behind forward at a short distance from the growing apex of the hypha, they are laid down irregularly throughout its length, dividing it into compartments which may contain living protoplasm or be empty of all contents. Sometimes a new branch may arise from below a septum, as shown in Pl. III, Fig. 4a. The septation increases as the culture ages and in old or badly nourished cultures is very free. In addition to complete partitions, isolated nodules, spines and plates of cellulose are laid down very commonly on the inside of the hyphal wall. Some of these may be seen in Pl. III, Fig. 8a. It naturally suggests itself that such cases as these represent early or arrested stages in the formation of complete septa, the process being then of the type known as *Spirogyra* or *Allomyces*. In several cases, however, the septum appears first as a thin plate, such as that shown in *c* of the same figure, and it has not been possible to decide whether this is preceded by a stage of the type figured in *a*. The mature septum usually becomes much thickened, the most common form showing a conical thickening, which in septa dividing full from empty portions of a hypha is always directed to the full side (Fig. 8b). Sometimes there is a corresponding depression on the opposite side and in the earlier stages a pore has been distinctly seen in the centre of the septum. The conical thickening marks the subsequent closure of this pore. Septa often develop at the origin of a lateral branch (Fig. 8d). All the septa, as well as the hyphal walls and the sporangia, give typical cellulose reactions.

The mycelium which creeps on the surface of the medium, where a densely felted layer is often formed, is more freely branched

than the aerial hyphæ (Pl. III, Fig. 1). The hyphæ which penetrate the medium are still more branched and are much less regular in diameter than the superficial mycelium (Pl. III, Fig. 2). Their ultimate ramifications are much branched, irregularly swollen, for the most part densely filled with protoplasm and sparingly septate (Pl. III, Fig. 3).

The sporangia are borne both laterally and terminally on the aerial hyphæ (Pl. III, Fig. 4). As in those found naturally on the leaf, their stalks are as a rule much narrowed and they break off, with a portion of the stalk attached, very readily. Sometimes, however, especially in secondary sporangia formed by the germination of primary sporangia, the stalk differs little from an ordinary hypha (Fig. 7a). Occasionally the insertion is by a broad base or the sporangium may develop as a unilateral swelling in the course of a hypha (Fig. 7b, Fig. 6, upper sporangium). Fig. 5 shows a group of sporangia from a French-bean agar culture 20 days old. They are still less uniform in size and shape than those found on the leaf, measuring from 20 to 61 by 15 to 31 μ in diameter and varying from almost cylindrical to broadly oval or club-shaped. They are, however, more elongated on the whole than in any other species of *Phytophthora* hitherto recorded. The papilla is always short and blunt and the base is marked at the point of insertion on the stalk by a strong cellulose plug projecting into the sporangium.

Many of the sporangia developed in culture, fall off within the tube, and in the absence of sufficient moisture to allow of zoospore production, most of these germinate as conidia by putting out one or more germ-tubes. In older cultures, a considerable proportion of the growth consists of hyphæ which have arisen from the first formed spores. This secondary growth again bears sporangia and it is at least conceivable that under certain conditions there may be a continuous succession of the vegetative and asexually reproductive stages of the fungus in cultivated soils, not only in this species, but in *Phytophthora infestans* and other species whose persistence from year to year, without any form of resting-spore, having been discovered under natural conditions, is still difficult

of explanation. It is no longer widely believed that potato blight begins in the new crop by the extension of mycelium from the tubers, where it has remained in a dormant condition since the previous crop was lifted, into the young shoots, where aerial sporangia are developed. De Bary's¹ early experiments showed, no doubt, that this course is sometimes followed. Recent investigators² hold that it is certainly rare and insufficient to explain the normal occurrence of the disease in the field. Clinton further notes that the first spots develop in leaves in contact with the ground and believes that the primary attack comes by direct infection of the leaves from the soil. It is true that *Phytophthora* do not appear to have been cultivated successfully using sterilised soil as a medium, but sterilisation may injure the soil regarded as a culture medium. The allied genus *Pythium* is quite commonly present in garden soils but as the mycelium is easily overlooked, special methods are required for its isolation, and the same holds good for other genera.³

The germ-tubes which arise from sporangia in cultures grow not only from the papilla but, when more than one are present, from any part of the spore wall (Pl. III, Fig. 12). Frequently secondary or even tertiary sporangia are formed after limited growth of the tube (Pl. III, Fig. 13). Sporangia that germinate in this fashion are often traversed by cellulose walls dividing the cavity into two or more compartments.

Chlamydospores are frequently formed in culture. They are spherical, spore-like bodies with a smooth, amber-coloured wall which may be 3μ thick and which takes a clear orange-yellow colour when treated with Schulze's solution. They may be terminal or intercalary or formed by a lateral swelling of the hypha (Pl. III,

¹ De Bary, A. Researches into the Nature of the Potato Fungus (*Phytophthora infestans*). Journ. of Botany, n. s., V, 1876.

² Clinton, G. P. Downy mildew of potatoes. Conn. Exper. Sta. Report, 1905, p. 304. Pethybridge, G. H. Considerations and experiments on the supposed infection of the potato crop with the blight fungus (*Phytophthora infestans*) by means of mycelium derived directly from the planted tubers. Proc. Roy. Dublin Soc. XIII, n. s., No. 2, 1911. Jones I. R., Giddings N. J., and Lutman B. F. Investigations of the Potato Fungus (*Phytophthora infestans*). U. S. Dept. of Agri. Bur. of Pl. Indus. Bull. 245, 1912.

³ Jensen, C. N. Fungus flora of the soil. Cornell Univ. Agric. Exper. Sta. Ithaca, Bull. 315, 1912, p. 420.

Fig. 9). They vary from but little more than the breadth of the hypha, to over 30μ in diameter. They are developed both in the basal part of the superficial mycelium and also embedded in the depth of the medium. They are quite distinctive bodies, not resembling in any way either the sporangia or the sexual spores of the fungus. The only other species of *Phytophthora* in which bodies which can be accurately identified as chlamydospores have been found appear to be *Phytophthora Faberi* Maub., in which Rorer¹ has briefly described their characters and Petch² also appears to have seen them, and *Phytophthora parasitica* Dastur³, in which they closely resemble those of the present species. It is not impossible that the bodies described as parthenogenetic oospores in several species are really chlamydospores. Thus several of the figures of the "oospores" of *Phytophthora Faberi* given by Coleman⁴, especially Pl. XVIII, Fig. 8, probably represent chlamydospores. So also it is possible that some of the "parthenogenetic oospores" of *Phytophthora infestans*, described by American authors, belong to the same category. In *Pythium palmicorum* the "resting spores" were at first taken for oospores and only later was their true character made out⁵. This species occupies almost an intermediate position between the genera *Pythium* and *Phytophthora* and its resting spores are evidently homologous with the chlamydospores of the latter.

The shape, the thickness and colour of the wall and its reaction with Schulze's solution, the absence of a papilla and the position on the bearing hypha, serve to distinguish these bodies from sporangia. From sexual spores they are equally distinguishable by the insertion on the hypha, more often intercalary than terminal and

¹ Rorer, J. B. Pod Rot, Canker and Chupon-Wilt of Cacao. Bull. Trinidad Dept. of Agric., IX, No. 65, 1910, pp. 13 and 15.

² Petch, T. Cacao and Hevea Canker. Circ. & Agric. Journ. of Roy. Bot. Gard., Ceylon, V, No. 13, 1910, p. 153.

³ Dastur, J. F. On *Phytophthora parasitica* nov. spec., a new disease of the Castor oil plant. Mem. Dept. of Agric. in India, Bot. Ser. V., No. 4, 1913.

⁴ Coleman, L. C. Diseases of the Areca Palm, I, Koleraga. Bull. Dept. of Agric. Mysore State, Mycol. Ser., No. 2, 1910.

⁵ Butler, E. J. An account of the genus *Pythium*. Mem. Dept. of Agric. in India, Bot. Ser. I, No. 5, 1907, p. 82 and Bud-rot of Palms in India *ib.*, III, No. 5, 1910, p. 255.

never on a special lateral stalk, the absence of an oogonial wall distinct from that of the spore, the absence of the antheridium, which is large and persistent in the sexual stage, the frequent occurrence in the superficial mycelium, whereas oospores are only produced buried in the medium and the variation in size in the same culture, which is much greater than with the sexual spores.

Their germination has not been followed. That they do not form zoospores is probable from the absence of a papilla and the fact that empty cases were not seen. They seemed to germinate by putting out a germ-tube in some cases but it was difficult to be certain that these were not intercalary chlamydospores and the supposed germ-tube merely the continuation of the stalk.

Oospores have been produced in considerable numbers in some of the cultures. They have been found hitherto only in French-bean agar, and in cultures which all belonged to the same strain, i.e., arose from the same original parent. They continued to develop in sub-cultures carried on over a period of six months and equally whether grown for several generations on this medium or when grown for one or more generations on other media such as corn meal or prune-juice agar and again transferred to French-bean agar. They were produced at laboratory temperatures during the coldest and hottest parts of the year, in a range of temperature from 15° to 30°C., daily mean.

The oogonia are found only in the entirely submerged mycelium, generally in a layer about one-eighth of an inch from the surface and are at once recognisable by the prominent and characteristically shaped antheridium (Pl. IV), which persists for months. Their development presents points of great interest, which were detected in this species almost simultaneously with those of the oogonia of *Phytophthora parasitica* Dastur, with which they agree in their main features. The antheridium is first formed as a club-shaped swelling at the end or near the end of a hypha, possibly sometimes also by direct lateral swelling in the course of the latter. When mature it is cut off by a distinct septum to form the male cell. To it now grows a branch, usually a lateral outgrowth from

a neighbouring, but distinct, hypha. This is the oogonial origin. As the apex reaches the wall of the antheridium, it indents it and then penetrates into the antheridial cavity. Usually it pursues a straight course to the opposite wall, which it again pierces to emerge on the further side. Sometimes it bends within the antheridium so that the point of exit is not opposite that of entry. The free apex now at once swells up into the oogonial cell, the swelling sometimes extending back to a part of the stalk within the antheridium which is either depressed into a cup-shaped hollow in which the oogonium sits or broadened out where the stalk emerges (Pl. IV, Figs. 4 to 12). The oogonium remains closely applied to the antheridium at its basal part and is apparently fused with it over a considerable extent. When the oogonial cell has reached its full size it is cut off by a septum, which is generally formed in the part of the stalk within the antheridium (Pl. IV, Figs. 6 and 13). The contents now contract away from the oogonial wall to form the oosphere, which ultimately develops a thick wall and lies loose in the oogonium, which it does not completely fill. At what stage fertilisation occurs could not be determined, no fertilisation tube having been detected.

So far as could be determined the oogonium arises always from a different hypha from that which bears the antheridium. Sometimes, however, the stalks of both organs swell up and a tangled mass results which prevents their origin being ascertained (Pl. IV, Fig. 14). In most cases where the oogonial stalk could be followed back it arose as a lateral branch from a larger hypha. In one case (Pl. IV, Fig. 11) the oogonial stalk became swollen and gave off two short branches which pierced two antheridia and bore two oogonia.

After the oogonium forms, the wall of the antheridium thickens somewhat, without becoming coloured, and remains clearly visible for a long time. The oogonial wall also thickens, sometimes considerably, and takes a yellow colour. The oogonial stalk below the antheridium is less persistent and in many cases disappears so as to give the impression that the oogonium arises as an ingrowth

from the wall of the antheridium which, after traversing the cavity, emerges on the opposite side. In some cultures a sort of secondary thickening develops on the outside of the oogonium, the outline of which becomes indistinct and granular or roughened (Pl. IV, Fig. 14). A similar condition has been observed in *Phytophthora parasitica*¹ and *Ph. infestans*².

The oogonia are subglobose and measure on an average 29.5μ in diameter (limits 24 to 35μ). The oospores are almost exactly spherical and average 23μ in diameter (limits 20 to 28μ). In these measurements a few abnormally large oogonia, in which oospores were not developed but which had normal antheridia, are omitted. They measured up to 41μ in diameter. So also in one or two tubes some very small spores were found with oogonia only 19μ and oospores 15μ in diameter. These were colourless and were probably degenerate forms. The antheridia vary considerably in size, being from 7 to 13μ in their longest diameter.

Germination of the sexual spore has not been observed.

IV. INOCULATION EXPERIMENTS WITH PHYTOPHTHORA COLOCASIE.

Raciborski³ tried to infect healthy leaves of *Solanum tuberosum* and *Nicotiana Tabacum* with sporangia of the *Phytophthora* on *Colocasia*, but without success.

Sawada⁴ successfully infected Taro (*Colocasia antiquorum*) and "water-potato" (? *Colocasia antiquorum* var.) with sporangia taken direct from a leaf and germinated in distilled water. He failed to infect similarly "Hasu-imo" (? *Colocasia indica* Hassk.) and "Manshu-imo" (? *Alocasia macrorrhiza* Schott.), though some small non-spreading spots developed at the seat of inoculation.

On its proper host *Phytophthora Colocasiae* is highly infective. Inoculations on the leaves with living active zoospores in distilled

¹ Dastur, J. F. On *Phytophthora parasitica* nov. spec., a new disease of the Castor oil plant. Mem. Dept. of Agric. in India, Bot. Ser., V, No. 4, 1913.

² Clinton, G. P. Oospores of Potato blight. Conn. Exper. Sta. Report, 1909-10, p. 770.

³ Raciborski, M. Parasitische Algen und Pilze Javas, I, 1900, p. 10.

⁴ Sawada, K. Infection of Taro. Rep. from Special Reports of the Formosan Agric. Exper. Sta., II, 1911, p. 7 of the reprint.

water gave in some cases visible signs of infection within six hours. Few parasites are known which produce such rapid results as this. In twenty-four hours brown spots at the point of inoculation were well developed. On the second day the patches were larger and yellow drops of liquid were oozing out on their surface. On the third day sporangia were formed. Controls kept under similar conditions showed no sign of disease. The germ-tubes from sporangia which germinate direct as conidia were also found capable of penetrating the leaf, entering across or between the lower epidermal cells.

Infection from zoospores takes place as shown in Plate II, Figs. 2 and 3, which were taken 24 hours after inoculation. The germ-tube pierces the cuticle, frequently swelling up or running for a short distance in the thickness of the outer covering of the epidermis (Fig. 2). It then either enters the epidermal cell or passes down in the partition wall between two cells. In the mesophyll it pursues a strictly intercellular course, sending haustoria into the neighbouring cells. When fructification is about to commence hyphae from the intercellular mycelium of the mesophyll again penetrate the epidermal cells to reach the surface (Figs. 1 and 4). They may also, as shown in Fig. 1, emerge through stomata or at the junction between adjoining cells.

Inoculations were next undertaken on a number of plants known to be attacked by previously described species of *Phytophthora*. Excepting *Colocasia* none of the *Araceae* appear to serve as hosts for the genus, several species of which, however, have a wide range of hosts.

The following table gives the details of these inoculations, all of which were made from pure cultures by sowing some of the sporangium-bearing mycelium in distilled water and using the suspension for the inoculations as soon as the water contained plenty of swimming zoospores. The plants were grown in pots and the inoculations done by placing a drop of the suspension on the unwounded or wounded leaf or stem of the plant and covering with a bell jar. The drops generally persisted for at least 48 hours, which

is ample to allow of infection. Leaf inoculations were always done on both surfaces. The wounding, except where otherwise stated, consisted in scraping away the epidermis with a sterile knife. Controls were kept in every case, those of potato being previously wounded on the leaves, the rest unwounded. These were placed as nearly as possible under the same conditions as the inoculated plants, well watered on the green parts and covered by bell jars. All the inoculations in this table were done at laboratory temperatures, which ranged from about 25° to 30° at the time. At the latter temperature the sporangia were sown in cooled water to secure zoospore emission, which does not take place freely at temperatures approaching $30^{\circ}\text{C}.$ (see p. 241).

Inoculations with Phytophthora Colocasiae Rac.

Name of plant.	Previously known to serve as host for :—	Nature of inoculation.	Results.
<i>Solanum tuberosum</i> . A mature plant.	<i>Ph. infestans</i> , para- sitica.	One shoot inoculated on a leaf and at the apical bud. Un- wounded.	Negative.
Ditto	Several leaves, peti- oles and the apical bud inoculated. Unwounded. Also one wounded leaf.	Do.
Ditto. A young plant.	Several leaves inocu- lated. Unwounded. Also several wound- ed leaves and the young stem after removing the epi- dermis.	The wounded leaves developed a wet rot, which rapidly spread to the petiole, causing their collapse. On microscopic ex- amination hyphae of <i>Phytophthora</i> were observed penetra- ting the epidermis at the margin of the wound, both through stomata and directly across the cells, and were also seen within the leaf tissues. The wounded stem devel- oped a brown dis- colouration which did not extend. Un- wounded leaves gave negative results.

Name of plant.	Previously known to serve as host for :—	Nature of inoculation.	Results.
<i>Solanum Melongena</i> . 4 young plants in a pot.	<i>Ph. omnivora</i> var. <i>Areca</i> , <i>parasitica</i> , <i>Faberi</i> .	A number of unwounded leaves inoculated, also one at a perforating wound.	Negative.
<i>Lycopersicum esculentum</i> . A mature plant.	<i>Ph. infestans</i> , <i>omnivora</i> var. <i>Areca</i> , <i>parasitica</i> , <i>Faberi</i> .	One shoot inoculated on several leaves and petioles. Unwounded.	Do.
Ditto	Several leaves inoculated. Unwounded.	Do.
Ditto	Several leaves and petioles wounded and inoculated.	A brown dry rot developed in the neighbourhood of some of the wounds but this did not spread except in one leaf, which dried up slowly. Infection appeared to be usually limited to the wounded area. The petiole inoculations gave no results.
<i>Syringa vulgaris</i> . 2 small plants in pots.	<i>Ph. Syringæ</i> , <i>omnivora</i> , <i>parasitica</i> .	Unwounded leaves and buds inoculated.	Negative.
<i>Nicotiana Tabacum</i> . A mature plant.	<i>Ph. Nicotianæ</i> ..	Three young leaves and one older inoculated. Unwounded. Also one leaf at a perforating wound.	Do.
Ditto	Several unwounded leaves.	
<i>Jasminum pubescens</i> . A rooted cutting from a mature plant.	<i>Ph. Syringæ</i> known on <i>Jasminum nudiflorum</i> .	As in <i>Syringa vulgaris</i> .	
<i>Jasminum Sambac</i> . A rooted cutting from a mature plant.	Ditto ..	Do.
<i>Ricinus communis</i> . Seedlings in pots.	<i>Ph. parasitica</i> ..	The seedlings sprinkled with the suspension of zoospores.	Do.
Ditto Mature plants.	Unwounded leaves and stems inoculated.	Do.



Name of plant.	Previously known to serve as host for:—	Nature of inoculation.	Results.
<i>Opuntia Dillenii</i> . A young plant.	<i>Ph. cactorum</i> , <i>omnivora</i> var. <i>Arceae</i> , <i>Fuberi</i> and <i>Syringae</i> known on several cacti.	Stems and buds inoculated without wounding.	Negative.
<i>Lepidium sativum</i> . Seedlings in a pot.	<i>Ph. omnivora</i> . ..	The seedlings sprinkled with the suspension of zoospores.	Do.
<i>Eurothera bicauis</i> . Seedlings in a pot.	<i>Ph. omnivora</i> , <i>omnivora</i> var. <i>Arceae</i> , <i>Fuberi</i> , parasitica.	As in <i>Lepidium sativum</i> .	Do.
Ditto	Ditto ..	Do.
<i>Clarkia elegans</i> . Seedlings in a pot.	<i>Ph. omnivora</i> , <i>omnivora</i> var. <i>Arceae</i> , <i>Fuberi</i> , parasitica.	Ditto ..	Do.
<i>Salpiglossus variabilis</i> . Seedlings in a pot.	<i>Ph. omnivora</i> , <i>omnivora</i> var. <i>Arceae</i> , <i>Fuberi</i> , parasitica known on <i>Salpiglossus</i> spp.	Ditto ..	Do.
<i>Schizanthus retusus</i> and mixed spp. Seedlings in a pot.	<i>Ph. infestans</i> , <i>omnivora</i> , <i>omnivora</i> var. <i>Arceae</i> , <i>Fuberi</i> , parasitica known on <i>Schizanthus</i> spp.	Ditto ..	Do.
<i>Fagopyrum esculentum</i> . Seedlings in two pots.	<i>Ph. omnivora</i> , parasitica known on <i>Fagopyrum</i> spp.	Ditto ..	Do.
<i>Gilia nivalis</i> and mixed spp. Seedlings in a pot.	<i>Ph. omnivora</i> , parasitica known on <i>Gilia</i> spp.	Ditto ..	Several seedlings drooping after three days. Placed in water on a slide gave a copious growth of <i>Phytophthora Colocasiae</i> , bearing sporangia in 24 hours. Both the cotyledons and the hypocotyl were infected, the latter being as a rule more severely attacked.
Ditto	Ditto ..	Several seedlings showed drooping cotyledons after two days. On the third day a number had collapsed. About 60 or 70% of these inoculated died. Hyphae with sporangia of <i>Phytophthora Colocasiae</i> developed in from 6 to 24 hours, when placed in water.

All the inoculations failed except those on seedlings of *Gilia*, where they succeeded well, and on wounded leaves of potato and tomato. The potato infection was definite, that of tomato less so, as little spreading took place. The controls remained healthy in every case. It is clear that the present fungus is restricted in its choice of hosts and therefore more highly specialised than species such as *omnivora* and its allies.

A few tests were made to determine whether temperature had any effect on the infection of seedlings. Seedlings of *Gilia*, *Clarkia*, *Oenothera* and *Fagopyrum* were used. Small pots containing the seedlings were placed in the cool incubator at 25°C., others being kept in the laboratory, the temperature of which at the time was just over 30°C. Both sets were inoculated with swimming zoospores, which at the former temperature are formed freely but at the latter only if sown in chilled water. The *Gilia* seedlings were attacked both in the cold and warm pots. *Oenothera* and *Clarkia* remained healthy in both. *Fagopyrum* was attacked at the cotyledon of one seedling only, in the cool incubator, but not at all in the warm pot. Temperature variations within the limits which markedly affect zoospore formation, have therefore little influence on the resistance of seedlings of the species tried, to *Phytophthora Colocasiae*.

V. SYSTEMATIC POSITION OF PHYTOPHTHORA COLOCASIAE.

Raciborski (*l.c.* p. 10) noted that this species appeared to be most closely allied to *Ph. Phaseoli* Thaxt. which it resembles in the size of the sporangia while differing in the characters of the stalk. With *Ph. omnivora* de Bary it agrees in the small number of sporangia formed on each sporangiophore. It is quite different from *Ph. Nicotianae* Breda de Haan.

Sawada (*l.c.* p. 1) transferred it to the genus *Kawakamia* as *K. Colocasiae* (Rac.) Sawada. *Kawakamia* is defined by Miyabe¹ as parasitic, without haustoria, with conidiophores emerging from the

¹ Miyabe, Kingo, in Kawakumi, T. "Kawakamia, a new genus belonging to the *Peronosporaceae*," Tokyo, Shokwabo & Co., 1904.

stomata, never branching from the base of the conidium and terminating with a short slender pedicel-cell; the conidia lemon-shaped with a prominent obtuse beak and falling with the thickened septum between the conidium and the pedicel-cell which forms a peculiar short tail to the spore. The other characters are as in *Phytophthora*, from which genus, according to Miyabe's description, it differs only in the pedicel-cell, a tail-like appendage to its conidium, and in the shape of the conidia and conidiophores. These differences appear to be of very slight systematic value and whatever may be said for Miyabe's type, *Kawakamia Cyperi*, the fungus now under consideration can scarcely be removed from *Phytophthora*. In mycelial characters, haustoria, sporangiophores and sporangia there are no points which cannot be paralleled amongst other *Phytophthoras*, excepting that the pedicel-cell may be of unusual length. It is, however, often very short in culture and such short stalks, remaining attached to the sporangium, are sometimes found in *Ph. infestans*¹ and *Ph. Phaseoli*². The characters of the sexual reproduction, unknown to Sawada, are equally in close similarity to other *Phytophthoras*, such as *Ph. parasitica*. Hence it is not possible to maintain Sawada's nomenclature and the species should be restored to the position given it by Raciborski.

Amongst other *Phytophthoras*, only a few show characters similar to those of *Ph. Colocasiae*. Of these the chief is *Ph. Phaseoli* Thaxt., a species parasitic on *Phaseolus lunatus* in the United States and Russia. From an examination of Clinton's³ figures it can hardly be doubted that the peculiar type of development of the sexual organs described above in *Ph. Colocasiae*, and also by Dastur⁴ in *Ph. parasitica*, is also found in *Ph. Phaseoli*. Clinton, indeed, states that for a long time it was difficult to decide whether or not the oogonial thread did not actually penetrate the antheridium

¹ De Bary, A. Die gegenwärtig herrschende Kartoffelkrankheit, Leipzig, 1861, figs. 4, 5 and 9.

² Clinton, G. P. Downy Mildew of Lima Beans. Conn. Exper. Sta. Report, 1905, p. 288.

³ Clinton, G. P. Artificial cultures of *Phytophthora*. Conn. Exper. Sta. Report, 1907 08, Plates LXXIV and LXXV.

⁴ Dastur, J. F. On *Phytophthora parasitica* nov. spec., a new disease of the Castor oil plant. Mem. Dept. of Agric. in India, Bot. Ser., V, No. 4, 1913.

and that he was not certain that this does not sometimes occur. The sexual organs themselves approach those of *Ph. Colocasia* closely in measurements. So also the absence of branching in the conidiophores is a point of resemblance. But the characters of the external growth on the host differ considerably, that of *Ph. Phaseoli* being conspicuous and forming a white felt on the pods while the *Colocasia* fungus is with difficulty visible to the naked eye. The conidiophores of the former are also many times longer than those of the latter and they produce several sporangia successively, the stalk continuing its growth by a new branch just below the spore, whereas on *Colocasia* usually only one spore is borne on each stalk. The sporangia are shorter in *Ph. Phaseoli* and the basal stalk left attached to the spore, when it is shed, is less distinct. These differences are enough to separate the two species.

From the other species of *Phytophthora* with sexual spores resembling ours, *Ph. parasitica* Dastur is at once separated by its broader sporangia, longer conidiophores and smaller oospores. Inoculation experiments also show that its range of hosts is very much wider. In the group of forms formerly included as *Ph. omnivora* de Bary, two require consideration as having possibly a similar type of development of the sexual organs to our species. These are *Ph. Fagi* as described by Himmelbaur¹ and *Ph. omnivora* var. *Arecae* Coleman. *Ph. Fagi* as first described by Hartig² was figured with the antheridium sometimes arising on a distinct hypha and attached to the oogonium at some distance from its base, sometimes on the same stalk and closely applied to the base, so that the oogonium appears to arise directly from the antheridium itself (Hartig *l.c.* p. 49 and Taf. III, fig. 24 b.). Himmelbaur states that the antheridium is attached to the underside of the oogonium, near its base. It appears probable that a penetration of the antheridium by the oogonial stalk sometimes occurs in

¹ Himmelbaur, W. Zur Kenntnis der Phytophthoren. Jahrb. d. Hamburg Wissensch. Anstalten, XXVIII, 1910.

² Hartig, B. Der Buchenkeimlingspilz, *Phytophthora Fagi*. Unters. a. d. forstbotan. Inst. z. München, I, 1880.

this species. It is, however, readily distinguished from *Ph. Colocasiae* by its regularly shaped and smaller sporangia. In the species on *Areca*, as described by Coleman¹, the antheridium arises, in some cases at least, before the oogonium and the latter comes to lie immediately above the antheridium, which is applied at its base. His figures suggest an actual penetration of the antheridium by the stalk of the oogonium (see especially Pl. XVII, Fig. 9 and Pl. XVIII, Figs. 1 and 3). But the oospores are larger than in *Ph. Colocasiae* and the sporangia broader and borne on more branched conidiophores, which form a dense felt on the nuts. The range of hosts is also considerably wider.

The only other species in which it appears likely that the oogonial origin grows through the antheridium is *Ph. infestans*². Clinton's figures, especially Pl. XXXIX E and Pl. XI, B, E, F and I, are clearly capable of this interpretation. But the oospores are larger than in *Ph. Colocasiae* and the sporangia smaller and borne on characteristically branched conidiophores and there is no danger of confusing the two species.

In none of the other species of *Phytophthora* which have been described are marked relationships with *Ph. Colocasiae* evident, either in the sexual or asexual stages. The species is indeed one of the most distinctive in the genus.

VI. TREATMENT OF THE DISEASE.

Sawada reports an experiment to test the effect of Bordeaux mixture in checking the disease. Half of an infected field was sprayed with Bordeaux mixture to which soap had been added. A month later the sprayed portion was found less infected than the remainder, although there had been much rain and continued gloomy weather. Before spraying all infected leaves were removed.

Bordeaux mixture has considerable prospects of success, owing to its proved efficacy in allied diseases. The sporangia are

¹ Coleman, L. C. Diseases of the Areca Palm, I. Koleroga. Bull. Dept. of Agric., Mysore State, Mycol. Ser. No. 2, 1910, and *Annales Mycologiques*, VIII, 1910, p. 591.

² Clinton, G. P. Oospores of Potato Blight. Conn. Exper. Sta. Report, 1909-10, p. 753.

produced chiefly on the upper surface of the leaves and are therefore more readily reached by the mixture than if they were confined to the under surface, as often occurs in potato blight. Experiments to test the value of spraying have not been possible, as the disease has not been severe at Pusa since 1909, when its study was commenced. The expense of spraying makes it a method of treatment which could only be practicable in very severe attacks and it is doubtful if it could be brought within the means of the ordinary cultivator in India.

In the early stages of attack, all spotted leaves must be removed and destroyed. This will be effective in delaying the period of severe attack and giving the corms time to form.

Corms kept for seed should be gone through at intervals and any that show signs of rotting, removed. At the time of planting only sound corms should be selected. This is probably the most important point in the control of the disease, as provided that a sufficient rotation is practised, the fungus is likely to disappear from the soil unless reintroduced in infected corms.

Planting in shady localities should be avoided, as there is no doubt that the severity of the attack largely depends on the humidity of the air.

December 1912.

DESCRIPTION OF PLATES I TO IV.

(*Phytophthora Colocasiae* RAC.)

(Plate I was painted by K. Das; the microscopical drawings were done in pencil with the aid of the camera lucida by E. J. B., and copied in ink by K. Das.)

PLATE I.

Lower figure: a leaf of *Colocasia antiquorum* Schott. severely attacked by *Phytophthora Colocasiae* Rac., viewed from above.

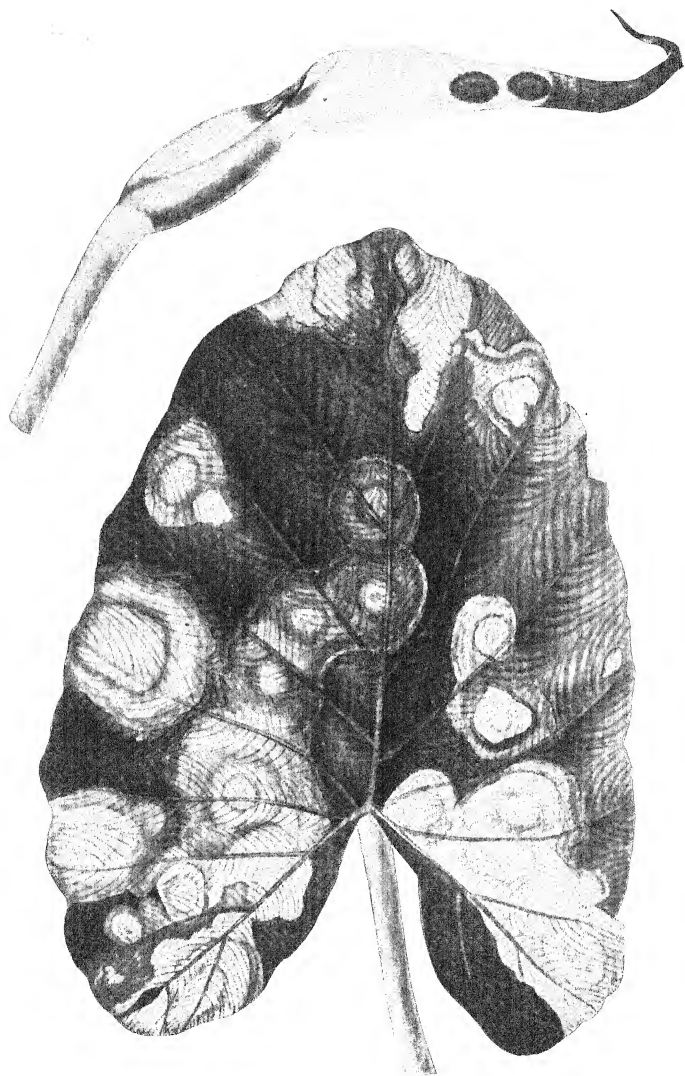
Upper figure: a young flower similarly affected.

PLATE II.

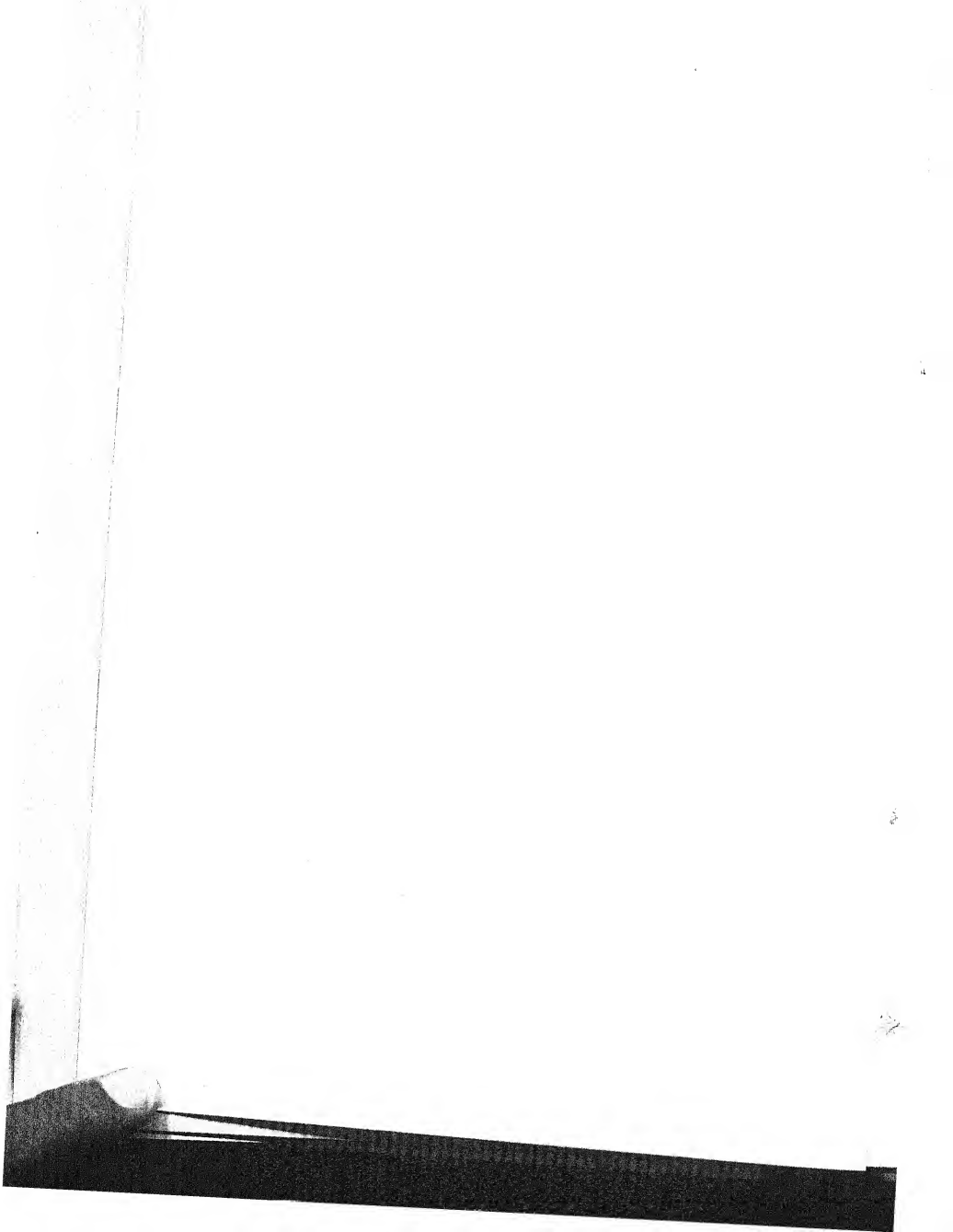
- Fig. 1. Surface view of a portion of a leaf of *Colocasia antiquorum*, bearing sporangia of *Phytophthora Colocasiae*. X 320.
Fig. 2. Infection of upper surface of *Colocasia* leaf by germinating zoospores, 24 hours after inoculation. X 930.
Fig. 3. Ditto, showing germ-tubes passing between the epidermal cells, not through them. X 930.
Fig. 4. Emergence of a hypha through an epidermal cell of the under surface of the same leaf as in Fig. 3, third day after inoculation. X 930.
Fig. 5. Part of a section of the corn of *Colocasia antiquorum*, showing hyphae of *Phytophthora Colocasiae* both in and between the cells of the parenchyma and also in the vessels. X 200.

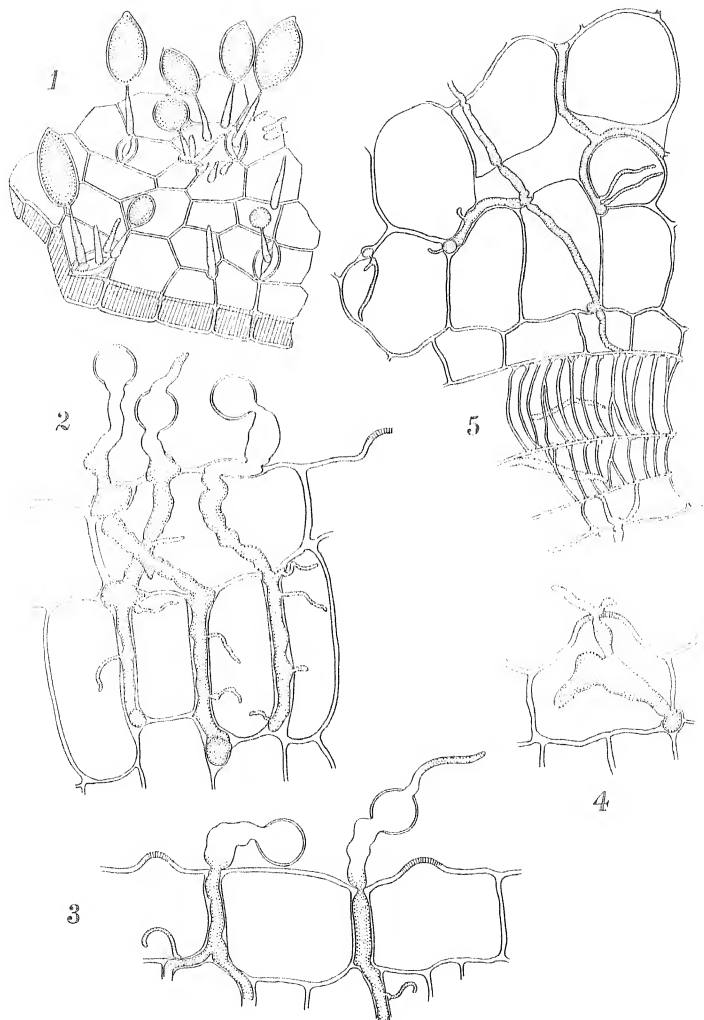
PLATE III.

- Fig. 1. Branching hyphae from a bean-agar culture at about the surface of the agar. X 330.
Fig. 2. Ditto from submerged mycelium. X 330.
Fig. 3. Ultimate ramifications of submerged mycelium in bean-agar. X 330.
Fig. 4. Aerial mycelium bearing sporangia from a bean-agar culture. The sporangia are both lateral and terminal. At *a*, a branch arises just below a septum. X 330.
Fig. 5. A group of sporangia from a strong bean-agar culture. X 330.
Fig. 6. Irregularly shaped sporangia, the upper formed without stalk by lateral outgrowth of a hypha. X 330.
Fig. 7. Two sporangia with unusual types of insertion. X 330.



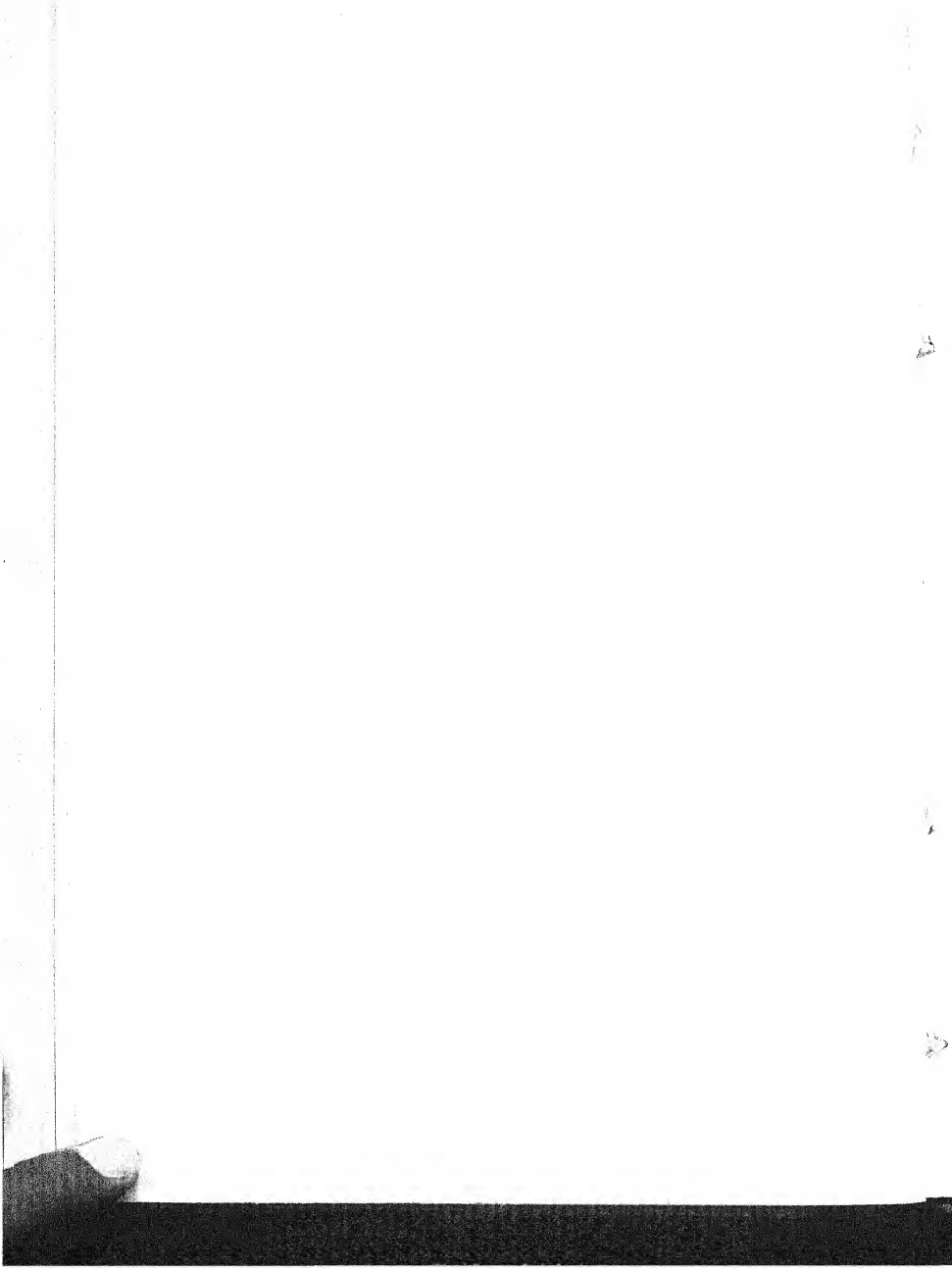
INFLORESCENCE AND LEAF OF COLOCASIA ATTACKED BY PHYTOPHTHORA.

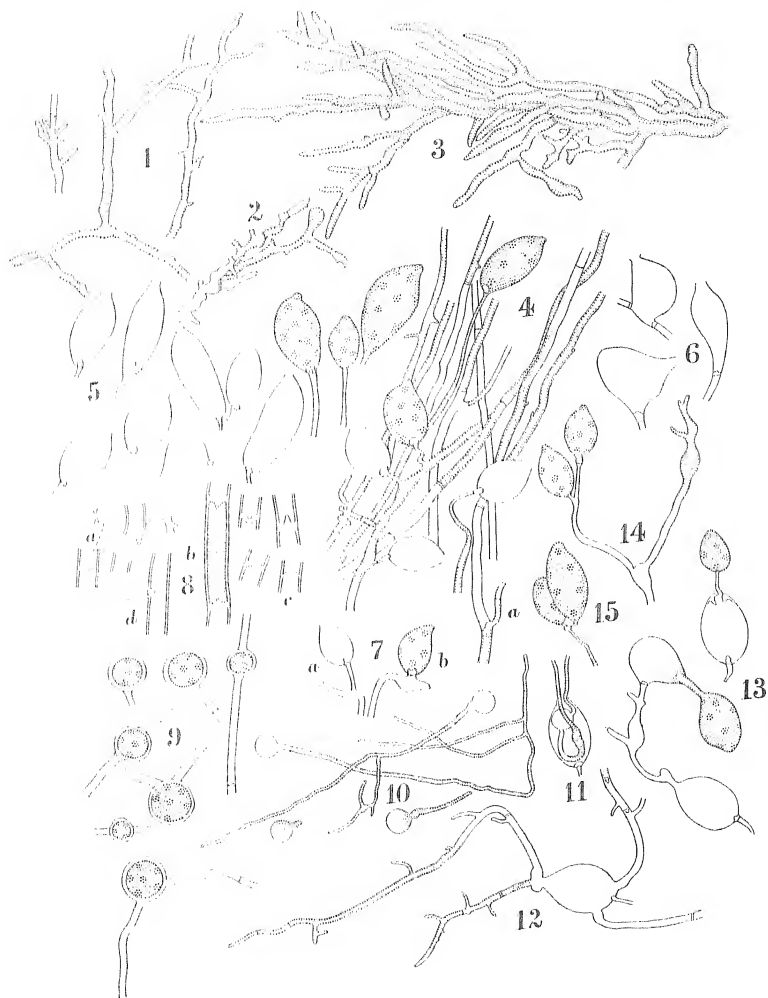




K. D. Das

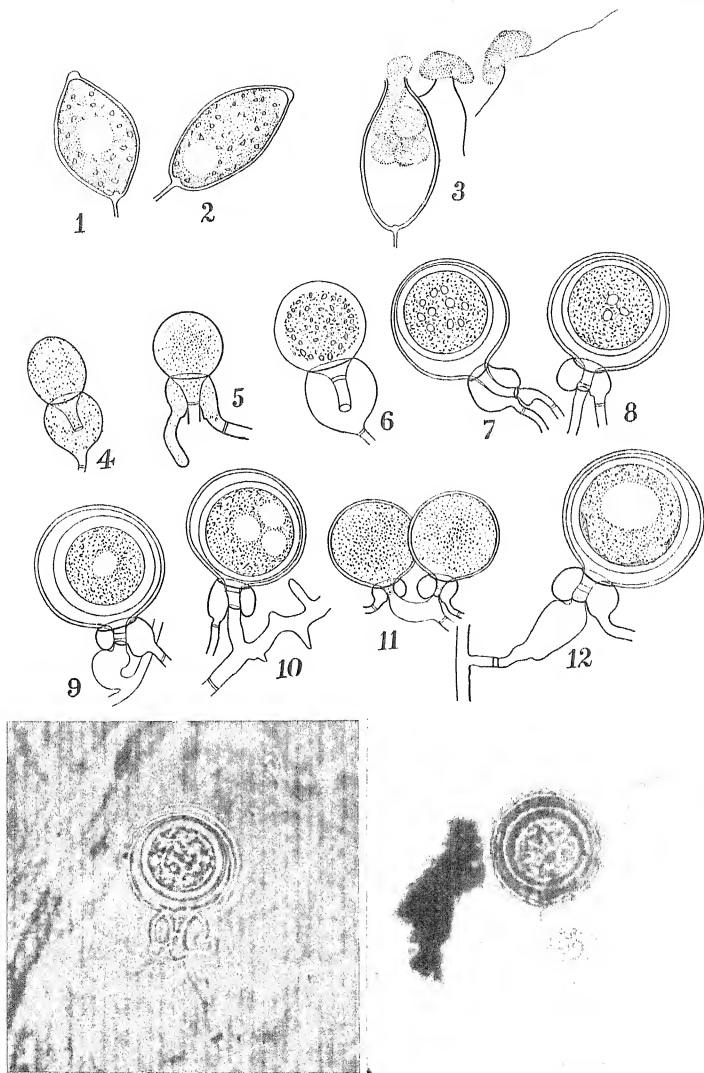
PHYTOPHTHORA COLOCASIAE RAC.





R. D. B. 1904

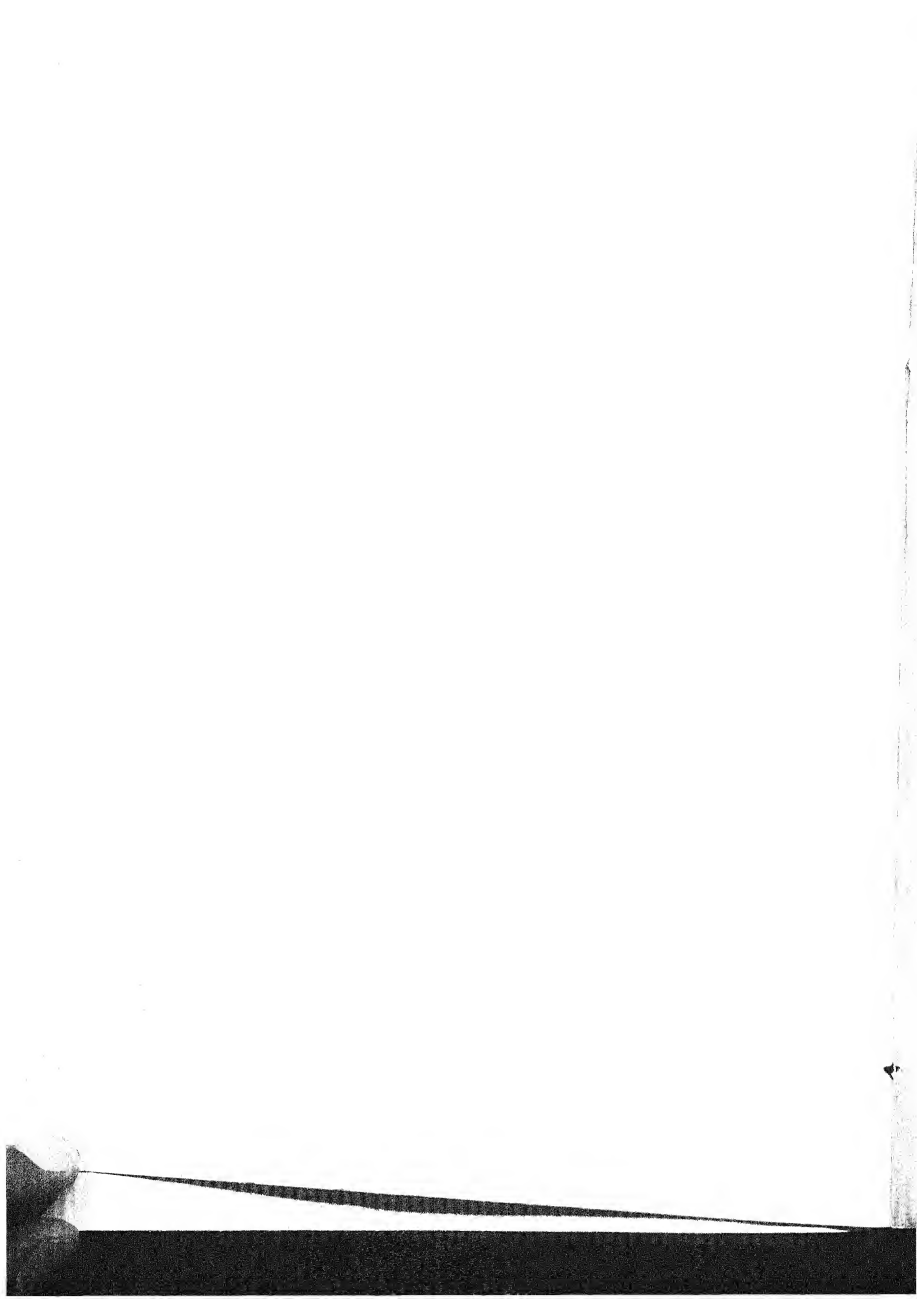
PHYTOPHTHORA COLOCASIAE RAC.



13.

PHYTOPHTHORA COLOCASIAE RAC.

14.



- Fig. 8. Details of the septa. *a*. A group showing the formation of cellulose processes on one or both sides of the hypha. *b*. Septa in an old hypha, the space between the septa being filled with cell contents, while above and below it is empty. *c*. A thin regular septum probably of very recent formation. *d*. Septum cutting off a lateral branch. The other septa show various types of thickening. X 930.
- Fig. 9. Chlamydospores from oat-juice agar. X 330.
- Fig. 10. Germination of the zoospores. X 330.
- Fig. 11. Germination of a zoospore while still within the sporangium. X 330.
- Fig. 12. Germination of a sporangium with direct production of hyphae.
- Fig. 13. Two sporangia which have germinated with production of secondary sporangia, the lower one having formed a tertiary also. X 330.
- Fig. 14. Hypha with sporangia from a corn taken about a month after harvest, cut in two and placed in a moist chamber. X 330.
- Fig. 15. Two sporangia arising from the same stalk, the lower formed after the upper, which it has pushed to one side. X 330.

PLATE IV.

- Fig. 1. A mature sporangium with single irregular vacuole and distinct papilla. X 630.
- Fig. 2. A sporangium shortly before discharge, showing the spherical vacuole. X 630.
- Fig. 3. Escape of the zoospores. On the right two free swimming zoospores. X 630.
- Fig. 4. Formation of the oogonium on a stalk which has penetrated the antheridium. X 930.
- Fig. 5. Ditto, later stage. X 930.
- Fig. 6. Ditto, later stage, the contents of the oogonium having contracted to form the oosphere. Antheridium empty. X 930.
- Figs. 7, 8, 9, 10 and 12. Fully formed oospores with persistent antheridia surrounding the stalks of the oogonia. The origin of the oogonium on a lateral branch from a hypha is seen in Figs. 9, 10 and 12. X 930.
- Fig. 11. Two oogonia on a common stalk, the branches from which penetrate two distinct antheridia. X 930.
- Fig. 13. Mature oospore, showing persistent antheridium surrounding the stalk of the oogonium. The antheridial stalk is visible on the right. Micro-photograph.
- Fig. 14. Ditto, showing type of oogonium with thickened wall. Micro-photograph.

(We are indebted to Mr. C. M. Hutchinson, Imperial Agricultural Bacteriologist, Pusa, for the micro-photographs reproduced in Figs. 13 and 14, for which a Zeiss 3 mm. apochromatic oil immersion lens was used.)

PYTHIUM DE BARYANUM HESSE.

BY

E. J. BUTLER, M.B., F.L.S.

Imperial Mycologist.

Pythium de Baryanum Hesse, though extremely common as a facultative parasite in garden soils in Europe and the United States, has not, so far as the writer is aware, previously been recorded in Asia. It has recently been isolated from the soil of the Pusa Farm and a short note on its occurrence may be not without interest.

The species was first described by Hesse¹ in 1874, as the cause of a disease, since widely known under the name of "damping off," of seedlings of *Camelina sativa*. It has frequently been observed since on many hosts, and a brief summary of our knowledge of its habits and the plants which it is capable of attacking was published in these Memoirs in 1907². More recently it has come into prominence in Central Europe as a cause of the disease of beet-root (especially sugar beet) known as "wurzelbrand"³.

In November 1912, some seeds of castor (*Ricinus communis*) which had been sown in pots of unsterilised Pusa soil and had failed to germinate, were examined. A Phycomycete, bearing numerous oospores, was found in the caruncle of the seed. Isolated on boiled ants in the manner described in the writer's memoir on *Pythium*, a copious clean growth was obtained in water. This

¹ Hesse, R. Ueber *Pythium de Baryanum*, ein endophytischer Schmarotzer, Halle (Inaugr. Dissert.), 1874.

² Butler, E. J. An account of the genus *Pythium* and some *Chytridiaceae*, Mem. Dept. of Agric. in India, Bot. Ser., I, No. 5, 1907.

³ Peters, L. Ueber die Erreger des Wurzelbrandes, in Busse W. Untersuchungen über die Krankheiten der Rüben, 5, Arb. Kaiserl. Biol. Anstalt für Land- und Forstwirtschaft, VIII, 2, 1911, p. 211. (Gives a full account of the association of *Pythium de Baryanum* with this disease, including references to previous work.)

bore sporangia and conidia on the extra-matrical part of the culture and oospores within the tissues of the ant.

From an examination of these the fungus proved to be the well-known *Pythium de Baryanum*. Subsequent ant cultures gave oospores also on the extra-matrical mycelium. The fungus grew well on boiled *Ricinus* seeds but on this substratum few sporangia were formed, oospores being very numerous within and outside the tissues. Seedlings of garden cress (*Lepidium sativum*), which is well known to be subject to damping off, were attacked in characteristic fashion when grown in soil containing the fungus. The reproductive bodies produced in this substratum were chiefly oospores, which were developed both intra- and extra-matrically. The failure of several observers to obtain the sporangia of this species is possibly to be attributed to the cultures having been made on plant tissues only; sporangia appear to be rarely produced under such circumstances, whereas on animal tissues they are formed in great abundance.

The mycelium is composed of much branched hyphæ, the main strands being up to 4 or 5 μ . in diameter, the lateral ramifications much finer (Pl. V, Fig. 8). Irregular swellings, such as are formed in several other members of the genus, are not common, the hyphæ tapering regularly as a rule. Within the tissues, septa are infrequent, except in the older stages, but the aquatic mycelium is richly septate, especially as soon as the hyphal contents begin to be used up in the formation of the reproductive bodies (Fig. 9).

Sporangia are found in large numbers on the second day in ant cultures. They are terminal or intercalar, the former being mostly spherical, the latter elliptical or irregular (Figs. 8 and 10). The hypha near a sporangium is frequently emptied of its contents, the empty part being sometimes cut off from the full by a septum. Germination in undisturbed cultures generally occurs while the sporangium is still attached to the mycelium. A very prominent beak is formed, generally laterally and about equal to the diameter of the sporangium in length. The sides of this beak are not usually as straight as is figured by Hesse. A single large vacuole is

present in mature sporangia but disappears shortly before zoospore-formation. The latter process is exactly similar to that of other species of the genus, the sporangial contents escaping into a thin-walled vesicle formed at the apex of the beak and maturation of the zoospores taking place within this vesicle (Figs. 11 and 12). Five to eighteen or more zoospores may be given by a sporangium. The zoospores are still figured, by a curious case of persistence of error, in most text-books, as being pear-shaped and uni-ciliate. Even in a recent original study¹, Hesse's old figures of 1874 have been copied. As the fungus is prescribed as a type in the botany syllabus of many institutions and is included in several popular text-books, it is as well to be accurate on this point. When first liberated the zoospore is longer than broad, the two longer sides being unequal in length and the shorter of the two sometimes depressed in the centre, the shape being like a bean somewhat flattened vertically, and with one end more pointed than the other (Fig. 13). From the hollow or hilum two cilia arise and diverge: by their movements the zoospore swims rapidly. As it slows down it becomes shorter and thicker, the two cilia being still distinct. When it comes to rest the cilia are retracted, but their position is still marked for a time by two droplets, perhaps of a fatty nature (Fig. 14). No case was seen of any tendency of the zoospores to divide into two 1-ciliate secondary zoospores, as described by Atkinson² in *Pythium intermedium*. It is extremely probable, therefore, that Hesse's figures were based on erroneous observations, the more so that they have not been confirmed by others since. The type of zoospore in *P. de Baryanum* is that normal for the *Peronosporaceae* as a whole.

On germination the zoospore emits usually a single germ-tube, which grows to a considerable length and branches at the extremity. It is sometimes septate (Fig. 15).

¹ Peters, L. Eine häufige Stecklingskrankheit der Polargewächse. *Gartenflora*, 1910, Taf. 1582.

² Atkinson, G. F. "Damping off." *Cornell University Agric. Exper. Stat. Bull.* 91, 1895.

The conidia, which were especially formed in cultures on plant tissues, do not differ in any way from the sporangia, except in their mode of germination. Morphologically they, no doubt, represent sporangia functionally changed in colonising the land. Germination occurs by the protrusion of one or more germ-tubes directly from the conidium (Fig. 16).

The sexual reproduction is well known. Oogonia are formed usually at the ends of lateral branches, but are sometimes also intercalary. A study of the early stages shows that the antheridia are often formed almost simultaneously with the oogonia, by branching of a single hypha (Fig. 5). The oogonial stalk is frequently longer than that of the antheridium, but curved so that the tips of the two hyphæ come into contact (Figs. 4, 5). Sometimes the antheridial cell is cut off while the oogonium is still in free communication with the stalk hypha (Fig. 2). Usually there is only one antheridium, which arises from the same hypha as the oogonium or, less often, from a neighbouring but distinct hypha. Occasionally two antheridia are present and other observers have seen three. Whether all are functional is not known. Sometimes instead of the antheridium appearing on a distinct branch, a part of the stalk just below the oogonium becomes cut off to form an antheridial cell and fertilisation takes place across the basal septum of the oogonium. I have not observed this condition. The cytological details of fertilisation have been fully studied by Miyake.¹

The following are the measurements of the various reproductive bodies obtained in my cultures, after fixation with osmic acid and mounting in glycerine:—

Sporangia and conidia : 15 to 26 μ . (av. 18 μ).

Zoospores after coming to rest : 6 to 8 μ . (av. 7 μ).

Oogonia : on ants 15.5 to 21 μ . (av. 18 μ .); on *Ricinus* and *Lepidium* : 15 to 26 μ . (av. 21.2 μ).

Oospores : 12 to 20 μ . (av. 16 μ).

¹ Miyake, K. The Fertilization of *Pythium de Baryanum*. Ann. of Bot. XV, 1901, p. 653.

It is somewhat curious that damping off of seedlings has not been more frequently observed in India. The few cases which had previously come under the writer's notice were due to *Rhizoctonia*. It is possible that this pest of gardens and plant houses in temperate countries is kept in check by climatic conditions in the tropics, since its occurrence at Pusa shows that it is present in soils exposed to as high a temperature as is ordinarily found in the tropics. Its parasitism appears to be as strongly marked here as elsewhere, for not only did it cause germinated cress seedlings to damp off, but it prevented germination in seeds of *Ricinus* in a manner similar to that recorded by Peters¹ in seeds of beet-root.

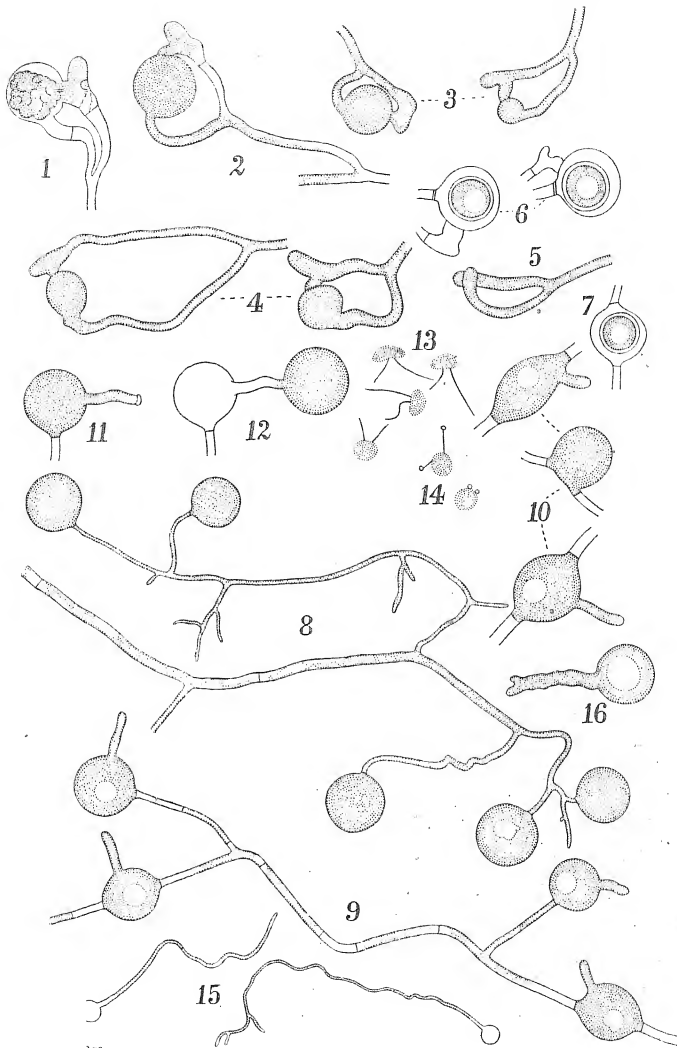
The treatment of damping off is based on the known conditions which predispose seedlings to this disease. These are overcrowding, growth in damp, stuffy localities, excess of water in the soil, excess of organic matter, especially decaying vegetable debris, and use of the same soil over and over again in plant houses and pots. The addition of sand to the soil, so as to improve its texture, frequent stirring of the top soil, exposure to sunlight and fresh air, evenness of temperature, good drainage and frequent change of soil, will generally suffice to keep seedlings in pots and forcing-beds healthy. In open cultivation the disease has not been found to do much damage, except in the case of beet-root, where several other parasitic fungi aid *Pythium de Baryanum* in causing the condition known as "wurzelbrand."

PUSA :

January 7th, 1913.

¹ Peters, L. Biol. Anstalt, VIII, 2, 1911, p. 225.





Das.

OVTHIUM de BARYANUM HESSE.

DESCRIPTION OF PLATE V.

(*Pythium de Baryanum* HESSE.)

(All figures magnified 640 diameters.)

- Fig. 1. Oogonium and antheridium during fertilisation.
Fig. 2. Oogonium and antheridium before fertilisation has commenced.
The antheridial cell already cut off, the oogonium still in communication with the stalk hypha.
Figs. 3 and 4. Early stages of oogonium and antheridium.
Fig. 5. Very early stage in the formation of the sexual organs. The more curved branch becomes the oogonium, the other the antheridium.
Fig. 6. Two ripe oospores, showing antheridia still attached.
Fig. 7. An intercalar ripe oospore.
Fig. 8. Mycelium with sporangia, before the commencement of germination.
Fig. 9. Older mycelium with sporangia which have commenced to germinate by putting out beaks.
Fig. 10. Intercalar sporangia, showing irregular shapes.
Fig. 11. Sporangium just prior to liberation of zoospores. The vacuole has disappeared and the tip of the beak is furnished with a hyaline cap.
Fig. 12. The same sporangium a few minutes later. The hyaline cap has blown out to a thin-walled bladder in which the protoplasm of the sporangium has accumulated.
Fig. 13. Zoospores during the free swimming period.
Fig. 14. Zoospores after coming to rest and while the two cilia are being retracted.
Fig. 15. Germination of the zoospores.
Fig. 16. A conidium shortly after germination. The germ-tube is beginning to branch at its tip and part of the spore-contents have passed into it.

OBSERVATIONS ON THE DOWNY MILDEW
(*SCLEROSPORA GRAMINICOLA* (SACC.)
SCHROET.) OF BAJRI AND JOWAR.

BY

G. S. KULKARNI, L.A.G.,

Mycological Assistant, Bombay Department of Agriculture.

THIS disease is found on three cereals, bajri (*Pennisetum typhoides*), jowar (*Andropogon Sorghum*), rala (*Setaria italica*), and a fodder grass (*Euchlena luxurians*). It was first described in India by Dr. Butler¹. He has dealt with it in detail, giving a full description of the fungus and the changes it induces in the hosts. My observations are chiefly concerned with the field characters of the disease on bajri and jowar, though certain additional interesting facts have been observed in connection with the conidial form on jowar.

BAJRI *SCLEROSPORA*.

The disease is detected on bajri plants in their very young stage, just after they put forth two or three leaves. The affected plants are pale yellow in colour, and the under surface of the leaves is covered with a white downy material, consisting of sporangiophores and sporangia. These are found on the upper surface of the leaves also to some extent. Ultimately, when the plant is mature, the peculiar malformed head is produced. For the detailed description of the inflorescence and the further effects of the fungus on the host, the Memoir already cited should be consulted. My observations are :—

- (1) That infection seems to occur only at or before the seedling stage of the plant, for the disease is confined to plants which show it from the beginning and the secondary

¹ Butler, E. J. Some diseases of Cereals caused by *Sclerospora graminicola*. Mem. Dept. of Agric. in India, Bot. Ser., II, No. 1, 1907.

infection of other plants in the field has not been observed. In order to verify this, 15 inoculations were made on plants not more than 20 days old. In each experiment the moving zoospores were transferred to healthy plants on both surfaces of the leaves and the plant was covered with a bell-jar. But there was no infection in any plant. Besides, in the fields it was observed that the plants in the neighbourhood of the affected one did not take disease, although the leaves of the healthy and the affected were touching each other.

- (2) The sporangial stage does not stop after the oospores have commenced to form. It can be found even at the time when the plant is completely mature, along with the oospore state.
- (3) Although the malformation of heads occurs only in the plants which are affected from the beginning, it is not uncommon to find plants otherwise apparently healthy producing deformed heads. In other cases a deformed axillary bud is the only sign of disease.

JOWAR SCLEROSPORA.

The disease manifests itself on this host in three different ways :

1st form :—Here the attack is just like that on bajri plants. The disease is found on the plant at a very early stage, just after it comes up from the soil. The leaves of the attacked plants are pale yellow in colour, the paleness extending from the base towards the tip, and they are narrow compared to those of healthy plants. Conidiophores and conidia are on both surfaces of the leaves but more abundantly on the lower surface. After five or six weeks, white streaks appear on the top leaves, indicating the beginning of oospore formation, and soon after leaf-shredding appears. The shredding usually begins from the margin towards the midrib from both sides of the leaf and, when complete, only the midrib

remains. The oospores are found even on the sheath of the leaf encircling the stem. The plant never attains the usual height and no head is produced. The disease is confined to the individual plants which are affected from the beginning.

2nd form :—The disease appears after the plant is about two months old. The top leaves of the affected plants become white, the whiteness extending to the base of the side leaves also. Irregular streaks appear on these white leaves, oospores are formed and soon shredding begins. On the lower leaves pale yellow patches appear at the base, on which the conidial stage is found. In this form the conidial stage follows the oogonial or, in some cases, they appear simultaneously. The affected plants rarely produce heads, and the heads, if formed, are much reduced in size and have a few small grains but are not malformed. It is the most common and prominent form in the fields and presumably causes considerable damage. Here also the disease is confined to the attacked plants and does not seem to spread from plant to plant.

3rd form :—The affected plants have on their leaves long, narrow streaks and patches. The streaks are first pale yellow in colour, become orange and finally turn dark brown after the cells of the attacked parts are dead. The conidial stage is found on both the surfaces of the patches, but more on the lower side. The patches appear to spread from the lower leaves to the upper leaves and from the apex of the leaf towards the base. The upper leaves have the patches only towards the tip, the middle ones have more than half of their surface covered with them, while the lower ones have their surface completely covered. The patches are bounded by prominent veins and increase mostly longitudinally. When the attack is severe and complete the whole leaf dries up after turning dark brown. No oospores are formed and no shredding of the leaf is observed. The plants produce normal heads. The disease seems to spread from plant to plant and in the field big patches of affected plants are visible.

From these three different modes of attack it seems that, in bajri and jowar, where the plants show disease from the beginning,

infection occurs at the seedling stage; while in the 2nd form of jowar attack, where it appears all of a sudden when the plant is half-grown, it recalls the mode of attack which occurs in some smuts, where the mycelium is in the grain, grows up in the young plant and appears in the head. In the third case of jowar attack the infection seems to be secondary, as it seems to spread from plant to plant.

Dr. Butler found the sporangial and oogonial forms of the disease on bajri but only the oogonial on jowar. The ripe fruits (oogonial forms) on bajri, and jowar do not differ much in size, shape and colour. According to his measurements the diameter of the oogonium on bajri is 42μ (average of 25 measurements), and that on jowar is 41μ , and on this basis he puts both the forms on jowar and bajri into one species. He says:—"In all systematic works which treat of *Sclerospora*, the characters of the ripe fruit are taken as the basis for classification, and on this basis it is impossible to separate the species" (on jowar) "from *Scl. graminicola*."

Although the oogonial forms do not differ, much difference is found in the conidial forms. There is not a great difference between the conidiophores as to their size, length, etc. In both I found as Dr. Butler described—"broad, rather short stalks, unbranched in the lower part, but usually with a few short thick branches dichotomously or trichotomously formed at the top, and crowned with numerous papillæ of characteristic shape, on which sporangia are borne." But these papillæ of the two show a remarkable difference in their length, the bajri ones being shorter than those of jowar. The maximum length of the bajri papillæ (measurement of 20) was 8.3μ , and that of jowar ones 16.3μ . The sporangia do not differ much in limits of size. The bajri sporangia vary from 19 to 31 by 16 to 21μ in diameter, and the jowar sporangia from 18 to 32 by 16 to 23μ in diameter. But the chief and constant difference is with regard to the shape of the conidia and the way they germinate. The bajri sporangia are broadly elliptical,—slightly pointed at the free end and germinate by liberating zoospores. The jowar sporangia are sub-orbicular, with no papilla at the free end and germinate like conidia

by a germ-tube and not by zoospores. These observations were made for two years, 1910 and 1911, at the kharif and rabi seasons, at different places and on several individuals of each host, and the differences were quite constant.

Further, I made cross-inoculations of both these *Sclerosporas*; bajri plants were inoculated with the conidia of the jowar *Sclerospora* and jowar plants with zoospores of the bajri *Sclerospora*. No infection took place in either case. In order to test this point further, a few affected young bajri plants were transplanted in a jowar plot and a few affected jowar plants in a bajri plot. In both the cases the affected plants grew with disease on them while their neighbours did not take the disease at all.

One reason for the failure of cross-inoculation might of course be that, as noted above, infection probably occurs at the seedling stage and not after. It is also possible that the two forms are each adapted to its own host like "biological forms."

These observations go to show that the bajri and jowar *Sclerosporas* are not one and the same fungus. They differ in their mode of attack and hence in the field characters of the diseases. Although the first form of attack, which occurs probably at the seedling stage, is common to both bajri and jowar, the 2nd and 3rd forms are peculiar to jowar alone. The malformations of the heads found in bajri never occur in jowar and the leaf-shredding which is so marked a character on jowar is much less common on bajri. Each form is not only fixed to its host but has modified morphologically also to a certain degree. These differences seem to me to justify us in regarding the two forms as two distinct varieties, if not species, and the following key is suggested for identification.

Sporangia broadly elliptical, with a papilla at the free end and germinating by zoospores *Sclerospora graminicola*.

Sporangia sub-orbicular, with no papilla at the free end and germinating as conidia by a germ-tube *S. graminicola* var. *Andropogonis* Sorghi.

The way in which the fungus lives from year to year is still a mystery. The conidia lose their vitality after three or four hours.

To test whether the mycelium of the fungus lives in the grain as some of the American mycologists believe, the grains of both jowar and bajri from attacked heads were sown in sterilised soil in pots. The plants did not show any disease and produced sound heads. Such grains were sectioned for mycelium and examined after staining in cotton blue. But no mycelium was seen. The obvious means by which it continues are the oospores. But they have resisted all my attempts to germinate them in the laboratory. As Dr. Butler says: "From the regular manner in which the disease appears in certain places every year, it is highly probable that germination occurs freely in nature under suitable conditions" and it would be possible to germinate them only when those conditions are reached.

Both the *Sclerosporas* occur in all places where the two crops are grown in the Bombay Presidency, with the exception that the jowar *Sclerospora* has not yet been observed in Gujerat.

POONA :

January, 1912.

DESCRIPTION OF PLATES VI AND VII.

(*Sclerospora graminicola* (SACC.) SCHROET).

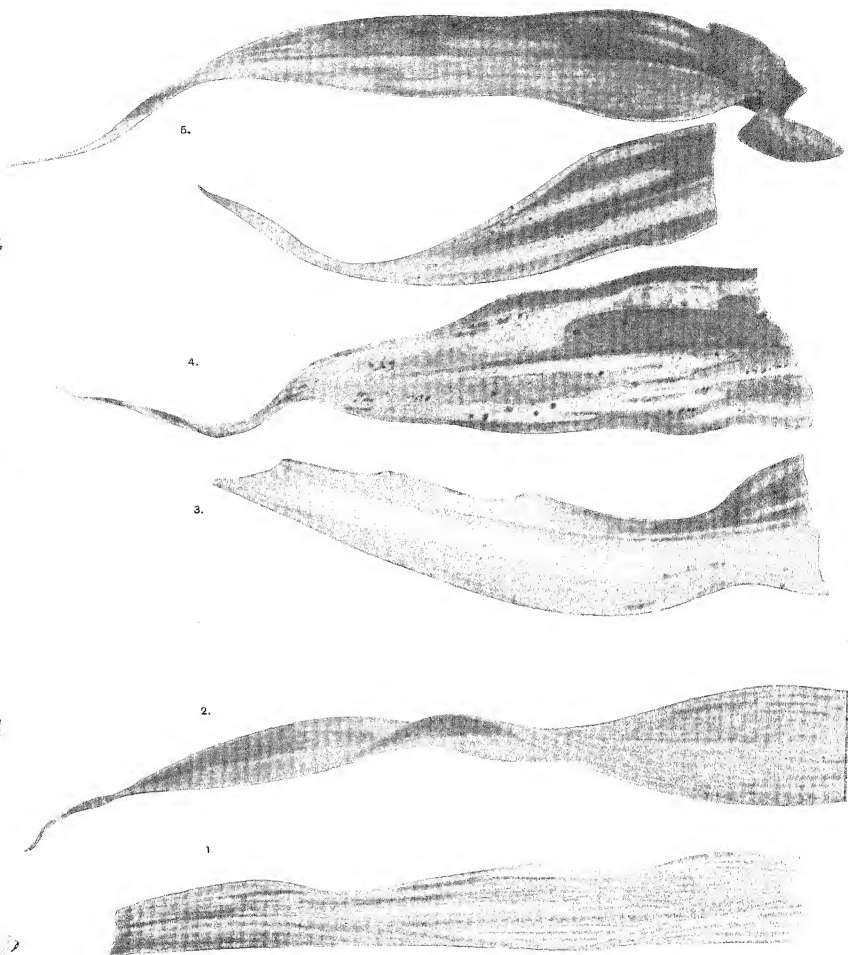
PLATE VI.

DOWNY MILDEW OF *Andropogon Sorghum*.

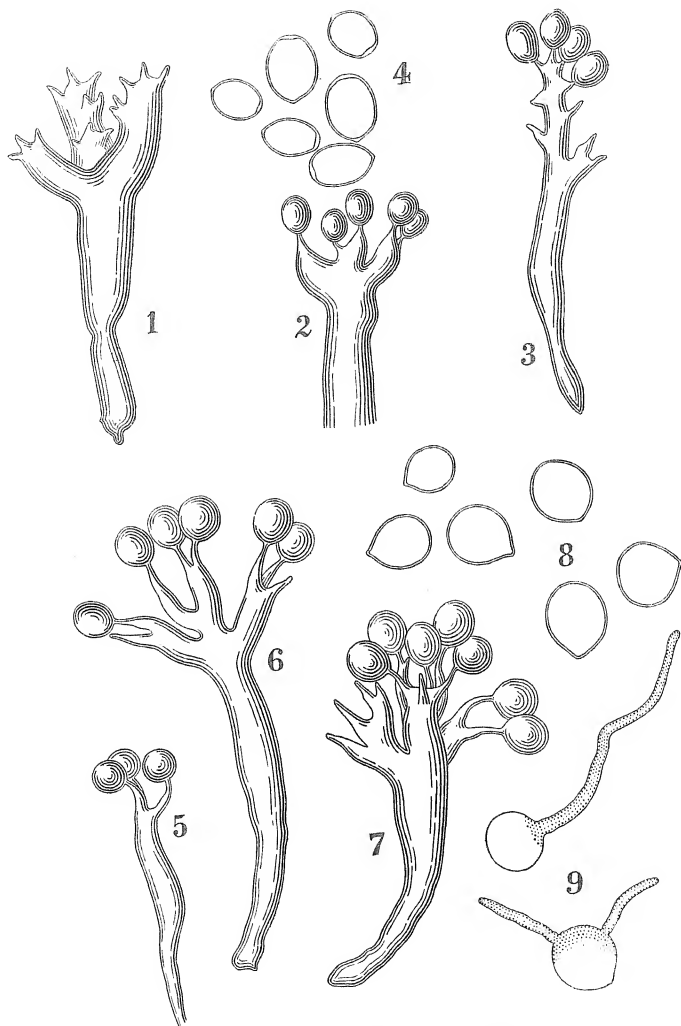
- Fig. 1. Second form, about the beginning of oospore-formation.
" 2. First form, early stage, upper surface of leaf.
" 3. Ditto later stage, lower surface of leaf.
" 4. Third form, late stage.
" 5. Ditto very early stage.

PLATE VII.

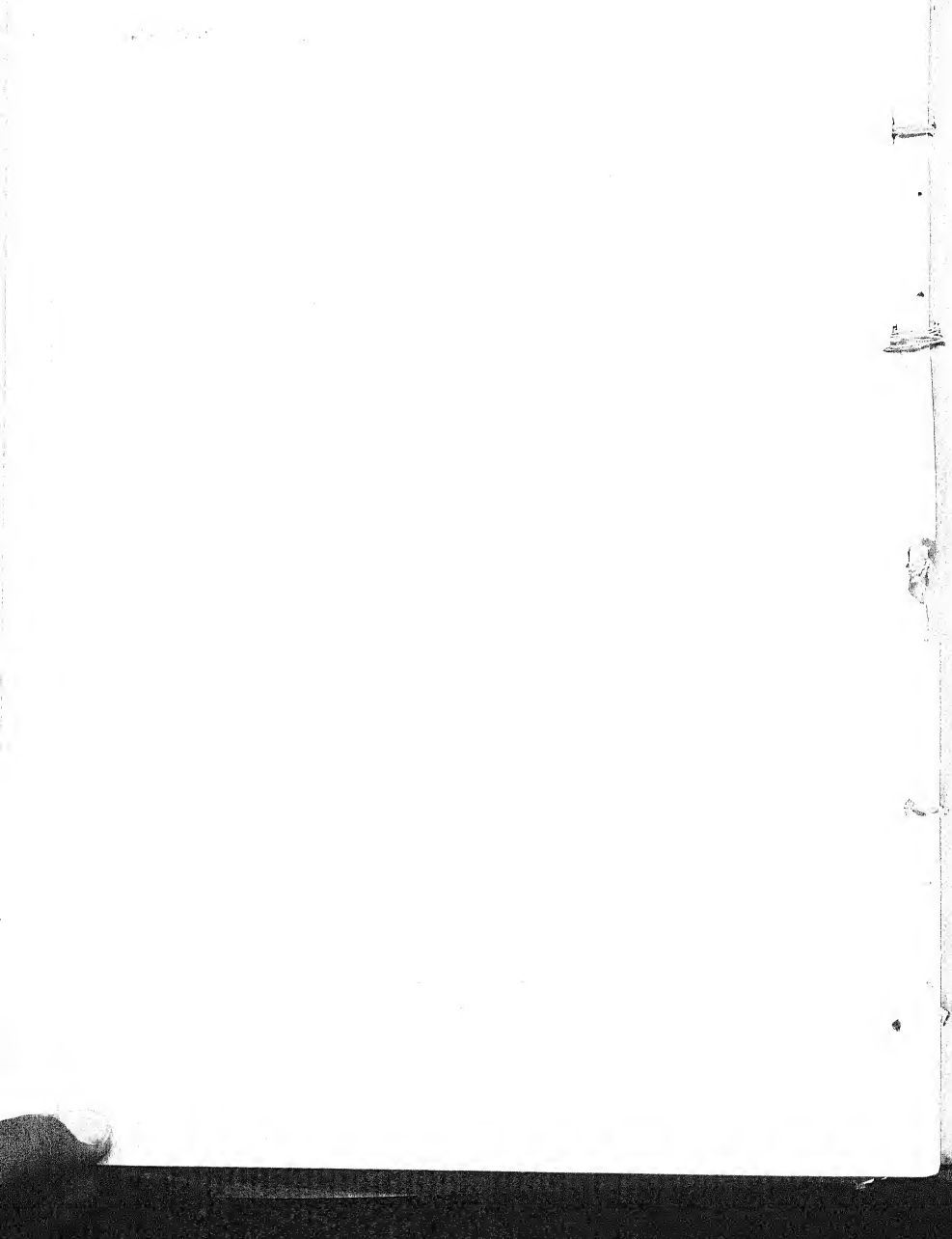
- Fig. 1. Conidiophore of *Sc. graminicola* from *Pennisetum*, showing the short papillae.
" 2. Early stage of development of the sporangia from *Pennisetum*.
" 3. Later stage of ditto ditto.
" 4. Sporangia from *Pennisetum*. Note the apical thickening which swells to form the papilla of discharge.
" 5. Early stage of development of the conidia of *Sc. graminicola* var. *Andropogonis Sorghi* from Sorghum.
Figs. 6 & 7. Later stage of ditto. Note the long papillae on which the spores are borne.
Fig. 8. Conidia from Sorghum.
" 9. Ditto germinating.



DOWNY MILDEW OF SORGHUM



SCLEROSPORAS OF PENNISETUM AND SORGHUM.



THE DOWNY MILDEW OF MAIZE

(*Sclerospora Maydis* (Rac.) Butl.)

BY

E. J. BUTLER, M.B., F.L.S.,

Imperial Mycologist.

A disease of maize, not previously known in India, appeared on the Pusa Farm last year (1912). It was found to be identical with one which causes great damage to this crop in Java, where it is termed by the natives "hijer" or "sleepy" disease. Raciborski¹, who first described it in 1897, prophesied its extension from Java to other maize-growing countries in the future; it is certainly of recent appearance at Pusa and it is probable that it has reached India from Java since Raciborski wrote. Up to the present it has not been recorded anywhere else, and we have no information as to its distribution in India or the rest of South-eastern Asia.

The disease becomes apparent before the plants have attained their full growth. The lower leaves are usually normal, but the upper part of the plant is chlorotic, owing to the disappearance of the leaf chlorophyll in long streaks (Pl. VIII). The growth of the plant is checked and the internodes frequently shortened so as to give a bunched appearance to the head (Pl. IX, Fig. 6). The affected plants are easily recognised at a distance, by their pale colour and stunted bunched growth. Such plants do not usually produce any grain, though sometimes small cobs are borne. The tassel or male inflorescence is more commonly developed and may be quite normal. The whole plant withers and dies, as a rule, some weeks

¹ Raciborski, M. Lijer, eine gefährliche Maiskrankheit, Ber. d. Deutsch. Bot. Gesellsch. XV, 1897, p. 475.

before the rest of the crop is ready for harvest. Raciborski describes the maize fields in Central Java as having thousands of such diseased plants, the disease being of epidemic intensity. At Pusa, so far, the cases have been few, but the progress of the disease will be watched with some anxiety as the crop is an extremely important one in Bihar and hitherto remarkably free from fungus diseases of any kind.

The cause is a fungus, named *Peronospora Maydis* by Raciborski. It is visible on cursory examination in the form of white downy or woolly patches on both surfaces of the chlorotic streaks on the leaves. These compose the fruiting or sporiferous stage of the fungus, the main body of which occurs as threads or hyphae within the tissues of the maize plant.

These internal hyphae are large, unseptate and pursue a tortuous course between the cells of the leaf mesophyll. Lateral swellings indent, and perhaps penetrate, the cell walls, acting probably as haustoria. When fructification is about to begin, hyphae collect in the sub-stomatal air-spaces and from them small clusters of conidiophores arise through the stomata, coming out on both surfaces of the leaf.

The conidiophores are very thick and rather short, being usually about 20 to 25 μ in breadth and 150 μ long, the length, however, varying considerably. They are usually unbranched at the base, but fork two or three times near the tip, the end branches being stout and provided each with two or more sterigmata (Pl. IX, Figs. 1 and 3). Each sterigma bears a single conidium, which is spherical when young but broadly oval when mature. The conidia fall off readily when ripe, leaving the conidiophores devoid of spores.

The conidia (Pl. IX, Figs. 1 and 3) are hyaline, thin-walled, not papillate or stalked and measure 28 to 45 by 16 to 22 μ in diameter. They germinate readily in water and may be found germinating in large numbers on infected leaves in the field. One or two germ-tubes arise from any part of the spore, giving a sparingly

branched, rather slender hypha, often of considerable length (Pl. IX, Fig. 4). The subsequent fate of the young plant developed from these spores is not known, the species not having been cultivated and no successful inoculations from conidia having been obtained. In hundreds of germinating spores observed, no case of germination by zoospores was seen.

Raciborski described a second spore form, which he stated was found only in the young stem and in the leaf-sheaths of young leaves. This he considered to be an oospore. Similar bodies were found in affected plants at Pusa and increased greatly in numbers as the plants decomposed. Fig. 5 of Pl. IX, shows the character of these bodies, agreeing in every respect with those described and figured by Raciborski. Further examination showed that they were the resting stage of a species of Protozoa allied to *Paramœcium*. This result was not unexpected, as the figure given by Raciborski is quite unlike any spore form known to occur in the *Peronosporaceæ*. A very thorough search was made for true oospores but none were found.

From the conidial stage, which alone is known with certainty, the fungus is a typical *Sclerospora*. It is quite unlike any *Peronospora*. In Berlese's Monograph¹ it is retained in *Peronospora*, as the author had not an opportunity of examining any specimens, but he states that, in his opinion, it is rather a *Sclerospora*. Pammel² has suggested that it is identical with *Sclerospora graminicola* (Sacc.) Schroet, the only *Sclerospora* so far known to possess a conidial stage. But a comparison with this species as it occurs in India on *Pennisetum typhoideum*, *Andropogon Sorghum* and species of *Setaria*³, shows a considerable difference in the size of the conidia, which are much larger on *Zea Mays* than on any of the others.

¹ Berlese, A. N. Saggio di una monografia delle *Peronosporaceæ*. Rivista di Patologia Vegetale, X, 1904, p. 219.

² Pammel L. H. Bull. Iowa Geological Survey, I, 1901, p. 188.

³ See Butler, E. J. Some diseases of Cereals caused by *Sclerospora graminicola*. Mem. Dept. of Agric. in India, Bot. Ser., II, No. 1, 1907, and the paper by Mr. G. S. Kulkarni in the present Memoir.

Sclerospora macrospora Sacc. occurs on maize and on many other grasses in Italy¹. This species is characterised by the absence of a conidial stage and the formation of large oospores within the affected tissues. Diseased plants can only be distinguished from healthy by malformations of the inflorescence, similar to those observed in *Pennisetum* and *Setaria* attacked by *S. graminicola*. There is no alteration in the colour, thickness or consistency of the leaves and no disintegration of the tissues such as causes the peculiar shredding of the leaves noticeable as a result of the action of *S. graminicola*. The symptoms described by Italian observers differ so essentially from those observed in Java and India that it appears to be unlikely that the same fungus can be the cause of both. It is possible, of course, that these differences are due to the occurrence of the fungus in two different stages in Europe and Asia and that, if the conidial stage of *S. macrospora* were to develop in Europe, the affected plants would resemble those observed by Raciborski and myself. Until true oospores are obtained in the eastern form, it will not be possible to decide this point, but in the meantime it appears to be improbable that the cause of "sleepy" disease is *S. macrospora*. It is stated in Duggar's "Fungus diseases of Plants" that *S. macrospora* has been reported on maize in the United States by Fairchild. I have not been able to find the reference and it is not included in Wilson's Host Index of North American *Peronosporales*².

With *S. graminicola* there are many points of similarity, especially now that a variety of this species is known which has lost the power of zoospore formation. The mildew of *Andropogon Sorghum*, in the 3rd form of attack described by Mr. Kulkarni in the preceding paper closely resembles that of maize. There

¹ See Cugini G. & G. B. Traverso. La *Sclerospora macrospora* Sacc. parassita della *Zea Mays* L. Stazione Sperimentali Agrarie Italiane, XXXV, 1902, p. 46. D'Ippolito, G. & G. B. Traverso. La *Sclerospora macrospora* Sacc. parassita delle infiorescenze virescenti di *Zea Mays* L. *ib.*, XXXVI, 1903, p. 975. D'Ippolito, G. Osservazioni intorno ad alcuni nuovi casi di frondescenza nelle infiorescenze di Granturco, *ib.*, XXXVIII, 1905, p. 998.

² Wilson G. W. Studies in North American *Peronosporales*, IV, Host Index. Bull. Torrey Bot. Club, XXXV, 1908, p. 543.

is the same copious production of conidia on chlorotic streaks on the leaves, the same absence of oospores, and the leaves do not split longitudinally, as they do in most cases of infection by *S. graminicola*. But the conidia differ so considerably in size and shape that it is not safe, in the absence of oospores, to consider the two forms to be merely varieties of the one species. For the present, therefore, it appears best to preserve the specific rank of the maize *Sclerospora*, as *S. Maydis* (Rac).

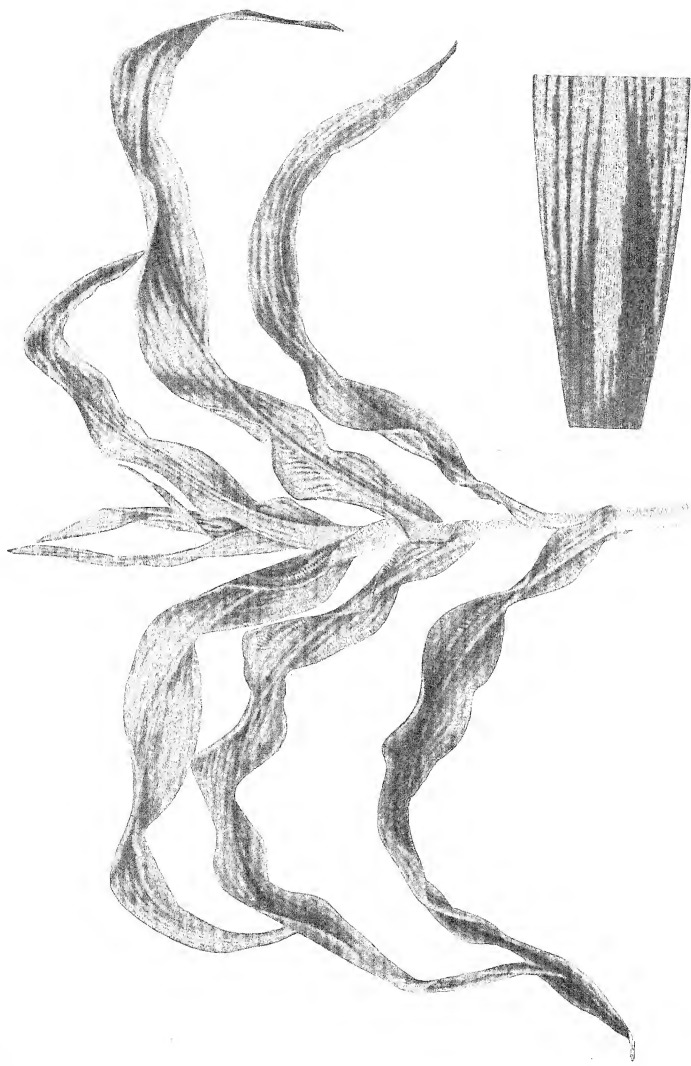
The life-history of the cereal downy mildews, which all belong to the present genus, is the most obscure amongst the *Peronosporaceæ*. Repeated attempts to germinate the oospore have failed. The conidia appear to be short-lived. The symptoms of the disease point to general infection of the plant, similar to what is known in the grain smuts, but whether this infection occurs subsequent to germination or results from mycelium already present in the grain at the time of sowing, is not known. Peglion¹ found the mycelium in the pericarp of wheat grains from deformed ears of plants attacked by *S. macrospora* and was able to trace its growth in plants developed from these seeds, but only when the grain was sown before it ripened fully. I have grown a considerable number of plants of *Pennisetum* from grain gathered from partly deformed ears, but without getting a single case of the disease. Kulkarni reports similar experiments with both *Pennisetum* and *Sorghum*, and neither he nor I have been able to find the mycelium in such grains. Further, I found several typical cases of the green ear disease of *Pennisetum* in a plot sown with grain heated by immersion in hot water to 65° C., for five minutes, a treatment known to be sufficient to kill the mycelium, and even the spores, of the grain smuts. It is probable, therefore, and Peglion himself is of the same opinion, that infection normally takes place after germination. This is most likely to occur through germination of oospores produced in the previous crop.

¹ Peglion, V. Ueber die Biologie der *Sclerospora*, eines Parasiten der Gramineen. Centralblatt für Bakteriologie, 2nd Ab. XXVIII, 1910, p. 580.

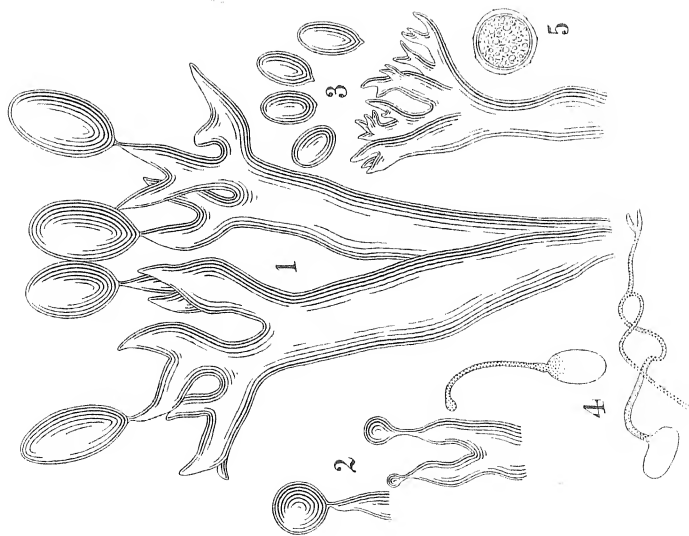
The treatment, on the information at present available, should be directed to preventing the persistence of the parasite in the oospore stage from the previous crop. For this purpose it will be sufficient to remove and destroy all diseased plants before they wither. Such plants can be easily recognised in the field and, so long as the disease remains sporadic, there should be no special difficulty in combating it.

PUSA :

January 15th, 1913.



DOWNY MILDEW (*SCLEROSPORA MAYDIS* (RAC.) BUTL.) ON MAIZE.



SCLEROSPORA MAYDIS (RAC.) BUTL.

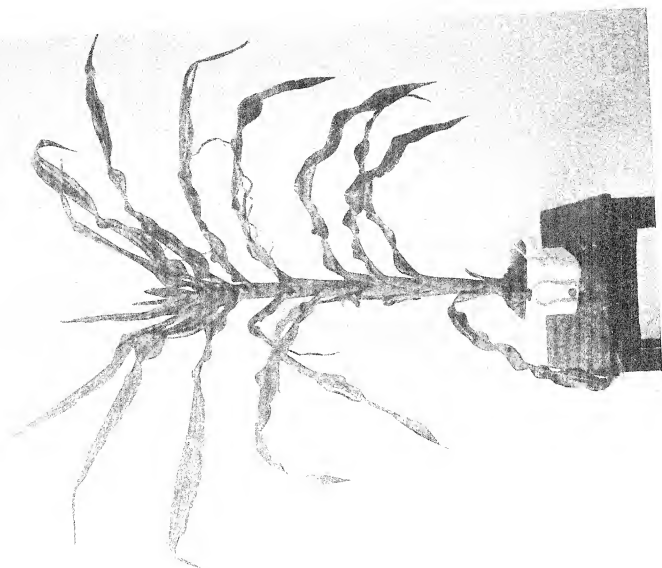


FIG. 6.
MAIZE PLANT AFFECTED WITH DOWNY MILDEW.

DESCRIPTION OF PLATES VIII AND IX.

(*Sclerospora Maydis* (Rac.) Butl.)

PLATE VIII.

Top of maize plant affected with downy mildew, reduced to two-ninths natural size, and part of leaf of same in early stage, reduced to one-third.

PLATE IX.

- Fig. 1. Two conidiophores, branched towards the top, the end branches provided with papillae, some of which bear spores. X 640.
- Fig. 2. Early stages of spore formation. The young conidium is spherical. X 640.
- Fig. 3. Much branched conidiophore with conidia which have become separated while mounting. X 320.
- Fig. 4. Two germinating conidia. X 320.
- Fig. 5. Cystic stage of a Protozoon found in the old leaf-sheaths of maize plants and closely resembling the bodies stated by Raciborski to be the oospores of *Sclerospora Maydis*. X 640.
- Fig. 6. A maize plant affected by *Sclerospora Maydis*, showing the manner in which the leaves stand out stiffly from the stalk and the bunched growth at the apex. Note the tip of the tassel appearing between the topmost leaves.



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